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Antimicrobial Inhibitory Activities of Phenolic Extracts from Four Selected Soybean Hulls in Culture and Chicken Skin Model Systems, and Preliminary ACE-Inhibitory Activity

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Antimicrobial Inhibitory Activities of Phenolic Extracts from Four Selected Soybean Hulls in Culture and Chicken Skin Model Systems, and Preliminary ACE-Inhibitory Activity

A thesis submitted in partial fulfillment
of the requirement for the degree of
Master of Science in Food Science

by

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ABSTRACT

Soybean components provide health benefits to humans. Soybean hulls, a major by-product of the soybean processing industry consist of complex carbohydrates, proteins, lipids and polyphenols such as anthocyanidins, proanthocyanidins and isoflavones. The polyphenolic compounds in the hulls give them various colors such as black, brown, green, yellow or even a mottled appearance. Studies have reported different soybean varieties with varying total phenolic compounds in their seed hulls, which have antioxidant property. Phenolic extracts can be used as substitutes for synthetic antimicrobials and preservatives to assist in preventing the growth of pathogens such as *Salmonella* Typhimurium, *E coli* 0157:H7, and *Campylobacter jejuni*, and work as angiotensin-converting enzyme (ACE) inhibitory. In this study we: 1) Prepared phenolic extracts from four selected colored soybean varieties, 2) Determined the minimum inhibitory effects of the extracts on *S. Typhimurium*, *E coli* 0157:H7, and *Campylobacter jejuni* in broth cultures, 3) Evaluated the inhibitory effects of extracts on *Salmonella* Typhimurium, *E coli*, and *Campylobacter jejuni* attached to chicken skin, and 4) Investigated the ACE-I inhibitory activity of the soybean hull phenolic extracts. The highest phenolic content was observed in R07-1927, the darkest colored soybean hull (4.29 mg CAE/g DW), and was found to be significantly different ($P < 0.0001$) from the conventional soybean variety, R08-4004 (1.63 mg CAE/g DW). For the antimicrobial activity of the extracts, a 3 day incubation with the phenolic extract from R07-1927 was found to produce 2 log reductions in *E coli* and *C. jejuni* counts, whereas a 6 day incubation was found to reduce *S. Typhimurium* and *E. coli* at 2 and 3 logs respectively. For the chicken skin study black soybean hull extract alone was used as it had the highest concentration of total phenolics. Log reductions of 1.39 was observed for *S. Typhimurium* and 1.24 for *E. coli* when inoculated chicken skins were incubated with the extract for 6 days. The results of our

study showed that soybean hull extracts may be used to reduce foodborne bacterial pathogens.

The preliminary study showed that the ACE-I inhibitory activity for the R07-1927 (black) phenolic extract was 52% while the R08- 4004 (yellow) extract showed 21%.

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CHAPTER 1

1.1 INTRODUCTION

Soybean components provide various health benefits due to their high protein and fiber content. Protein, oil, fatty acids, carbohydrates, isoflavones, and mineral contents determine the nutritional value of soybean seeds (Torres, Torre-Villalvazo & Tovar, 2006). In the United States, soybean ranks second among the most planted field crops with about 90% of its oil production coming from soybeans (Hamrick, 2016). In the state of Arkansas, soybeans stand as one of the top three cash crops of Arkansas farmers generating an income of about \$1.7 billion annually. Soybeans are on around 3.3 million acres of land with 41 out of 75 counties in the state cultivating the crop (Soybean Production in Arkansas, 2014).

The seed coat of soybeans known as soybean hull, is a by-product of the soybean oil and soybean meal industry, which constitute about 8% of the whole seed (Gnanasambandam & Proctor, 1999). Soybean hulls come in a variety of colors such as yellow, green, brown and black. The presence of polyphenols such as anthocyanins and proanthocyanidins are responsible for the different seed coat colors in soybeans (Todd & Vodkin, 1993). Polyphenols are secondary metabolites of plants, which are found in abundance in fruits, vegetables as well as legumes such as soybeans, and they impart color, flavor and sensory properties like sweetness, bitterness and astringency (Pratt, & Hudson, 1990; Cuppett & Schnepf, 1997; Floridi *et al.*, 2003). Studies have reported that different soybean varieties containing varying levels of total phenolics in their seed hulls, can supply excellent antioxidant and antimicrobial effects against several foodborne pathogens (Xu and Chang, 2008). Malenčić *et al* (2012) reported that all varieties of soybeans contain varying types of polyphenols such as anthocyanins and proanthocyanidins in abundance in dark colored hulls such as black and brown. Black soybeans have long been consumed as

significant source of food providing antimicrobial activity due to the rich phenolic content in their seed hulls (Xu & Chang, 2007; Astadi & *et al*, 2009). Additionally, research by Sebei *et al* (2013) showed that the phenolics in soybean were higher than in cowpea and mung bean sprouts.

Polyphenols are compounds possessing one or more hydroxyl groups attached to one or more aromatic hydrocarbon groups and form the most abundant antioxidants in human diet (Tapiero *et al.*, 2002). The polyphenols present in plants are involved in providing immunity or protection against ultraviolet radiation, different plant pathogens and parasites. Polyphenols are classified into different categories such as phenolic acids, flavonoids, stilbenes and lignans depending upon the number of phenol rings that they contain (Pandey and Rizvi, 2009).

Phenolic compounds from different plant sources have been studied for their wide spectrum of biological activities such as antioxidant, antimicrobial, and anticarcinogenic effects and so these can be used as replacement for synthetic compounds (Carbonaro *et al.*, 2002; Tapiero *et al.*, 2002; Floridi *et al.*, 2003; Nakamura *et al.*, 2003). Soybean hulls are a rich source of dietary fiber comprising of 86 % polysaccharides such as pectin, cellulose and hemicellulose along with polyphenols, phytic acid, vitamins and minerals (O'Bryan *et al.*, 2014). Studies by Xu & Chang (2007) shows that around 73 % of the total phenolics in soybeans are present in the seed coats or hulls.

Phenolics can be extracted from the hulls of soybeans using different solvents. The hulls of soybean need milling, grinding and sieving to obtain smaller particles of uniform size for efficient extraction of total phenolics. Solvents such as methanol, ethanol, acetone and ethyl acetate or their combinations with different proportions of water are used to extract total phenols from plant sources. Choosing the right solvent impacts the percentage of extracted phenolics (Abascal *et al.*, 2005; Xu & Chang 2007). For the extraction of phenolics from anthocyanin rich

plant sources commonly acidified organic solvents such as ethanol or methanol is used (Dai & Mumper, 2010). Methanol can be used to extract free and simple phenolics for analytical purposes, but it is not acceptable as a food grade solvent since it is considered as an environmental hazard due to its toxicity (Shi *et al.*, 2005). On the other hand, extraction of phenolics using ethanol is generally recognized as safe (GRAS) for human consumption (Li *et al.*, 2014). Several studies indicate that total phenolics as well as individual phenolic compounds extracted from different plant sources are able to inhibit foodborne pathogens (Madhavi *et al.*, 1995; Proestos *et al.*, 2005; Proestos *et al.*, 2006; Bolanho and Beléia, 2011; Junqueira-Gonçalves *et al.*, 2015).

Foodborne illness is a common public health problem worldwide with around 1 in every 6 person being affected in the United States each year. Consumption of poultry meat has been on the rise in recent years, which might have led poultry to be implicated in about 17-18 % of foodborne illnesses (Painter *et al.*, 2013). Raw poultry meat is often contaminated with bacteria such as *Salmonella*, *E coli*, *Campylobacter*, *Arcobacter*, *Listeria*, *Staphylococcus* and *Clostridium* (Doležalová *et al.*, 2010). Some of these bacteria such as *Salmonella* and *Campylobacter* are able to survive in the feather follicles or on the skin of poultry even after the different treatments during processing (Zhang *et al.*, 2013; Chaine *et al.*, 2013). Nearly 2.4 million cases are caused by *Campylobacter*, 1.4 million cases are caused by nontyphoidal *Salmonella* serovars, and 270,000 cases are caused by pathogenic *Escherichia coli*, including *E. coli* O157:H7 (mead *et al.*, 1999).

Raw chicken skin is vulnerable to contamination during their production, distribution and sale (Conner *et al.*, 2001). There are several ways in which contamination by these pathogens can occur including production, processing, distribution, retail marketing, handling and

preparation. Numerous epidemiological studies report that foods from animal sources are the main cause for foodborne disease (Todd, 1996; Petersen & James, 1998). Researchers have demonstrated effectiveness of an immersion in or spray of antimicrobials to reduce bacteria on chicken skin (Sukumaran *et al.*, 2015). Thus, varieties of chemical preservatives are used in the food industry to inhibit the growth of pathogenic bacteria. Phenolic extracts are used as substitutes for synthetic antimicrobials and preservatives to partially assist to prevent pathogen growth, and decrease the foodborne diseases (Microvet, 2011). While *Salmonella*, *E coli*, and *Campylobacter* are the main causes of foodborne illness from chicken which make them the organisms of greatest global concern, others include *Arcobacter*, *Helicobacter* spp (Cavitt, 2003). *Campylobacter*, *Salmonella*, and pathogenic *E. coli* are colonies that have a wide risk for human consumption, especially in chicken, and commercial chicken has been identified as the most significant source of these pathogens (Meng & Doyle, 1998). Studies worldwide reported that *Salmonella*, *E coli*, and *Campylobacter* are predominantly present in fresh meat and poultry (Todd, 1996).

Besides the antimicrobial, phenolics can work also as an Angiotensin-converting-enzyme inhibitor which is known to be responsible for causing hypertension, one type of heart disease (Ademiluyi & Oboh, 2013). More than 50% of the deaths for men and women cause are due to heart disease (CDC, 2015). Heart disease by hypertension is the number two killer in the U.S. (Farley *et al.*, 2010; Nwankwo *et al.*, 2013). It has been shown that antihypertensive activity connected inhibits the Angiotensin-I converting-enzyme (ACE-I) of catalytic activity (Lee *et al.*, 2010). ACE-I is known to be a major risk factor for causing hypertension (Liesmaa, 2010). ACE enzyme converts the hormone angiotensin I to the active vasoconstrictor angiotensin II by

removal of two C-terminal residues which lead to an increase in blood pressure (Maruyama & Suzuki, 1982).

Angiotensin-converting enzyme (ACE) inhibitors have the ability to block the enzyme which forms a substance for vascular constriction. Thus, blood vessels relax as well as decrease in blood volume, which leads to reduced blood pressure and prevents a heart attack or stroke (Drugs for hypertension, 2012). For ACE inhibitors there are many different medicines which help decrease the blood pressure, but at the same time they have side effects and interact with other medicines (Choi *et al.*, 2001; Hong *et al.*, 2008; García *et al.*, 2013). Recently, the search try to use the natural sources as alternatives to synthetic drugs which is great interest to prevent several side effects (Wijesekara & Kim, 2010). There are many diferent reports that soybean sources can inhibit the ACE-I (Chiang *et al.*, 2005; Yang *et al.*, 2011; Lassissi *et al.*, 2014). But the research in the use of phenolic extracts from different colored soybean hulls in inhibiting ACE -1 inhibition is very limied. Hence a preliminary study was conducted to determine the feasibility of phenoloc soybean hull extracts in the inhibition of ACE -1 inhibitors.

1.2 Hypothesis

Phenolic extracts from the hulls of soybean seeds can inhibit common foodborne pathogens such as *Salmonella* Typhimurium, *E coli*, and *Campylobacter jejuni*, commonly found on raw poultry as well as an affective ACE-I inhibitor.

1.3 The objectives of this study were to:

Objective 1: Prepare phenolic extracts from selected colored soybean varieties and quantify total phenolics.

Objective 2: Determine the minimum inhibitory effects of the extracts on *S. Typhimurium*, *E coli* 0157:H7, and *Campylobacter jejuni* in broth cultures.

Objective 3: Evaluate the inhibitory effects of extracts on *Salmonella* Typhimurium, *E coli*, and *Campylobacter jejuni* attached to chicken skin.

Objective 4: Conduct a preliminary feasibility study to investigate the ACE-I inhibitory activity of the extracts.

CHAPTER 2

2 LITERATURE REVIEW

2.1 Soybeans

2.1.1 Parts of soybean

Soybean seeds contain two parts: 1. the embryo comprising of two cotyledons which serves as the store house for protein and oil; 2. the seed coat (hull) which protects the cotyledons. The seed coat of soybeans also known as soybean hull, is a by-product of the soybean oil and soybean meal industry, which constitute about 8% of the whole seed (Gnanasambandam & Proctor, 1999). The cotyledons are the major constituents and make up 90% of the whole seed and the remaining 2% forms the embryonic axis (Oliveira *et al.*, 2007).

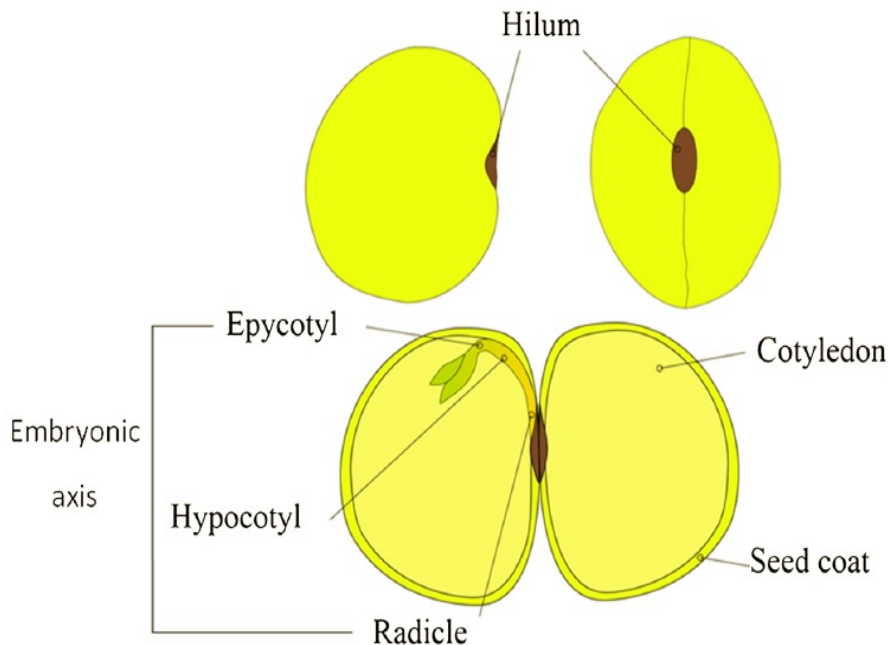


Figure 1. Soybean seed parts and installation (Medic *et al.*, 2014)

Soybean hulls are a rich source of dietary fiber comprising of 86 % polysaccharides such as pectin, cellulose and hemicellulose along with polyphenols, phytic acid, vitamins and minerals

(Liu *et al.*, 2013; O'Bryan *et al.*, 2014). The seed coats or hulls of soybeans have a variety of colors such as yellow, green, brown, black or sometimes a mixture of colors giving them a mottled appearance. Presence of polyphenolic compounds such as anthocyanins and proanthocyanidins in soybean hulls give the seed coats a variety of colors (Todd & Vodkin, 1993; Duenas *et al.*, 2006; Xu & Chang 2008; Malenčić *et al.*, 2012). However the yellow variety of soybeans was found to have not much of polyphenolics but was rich in isoflavones such as genistein (Todd & Vodkin, 1993; Malenčić *et al.*, 2012). Black soybeans have long been a part of Asian diet as a significant source of proteins in the seed and phenolic compounds in the seed coat (Xu, 2007; Astadi *et al.*, 2009).

2.1.2 Soybean production statistics:

Soybeans ranks second after corn among the most planted field crops (Hamrick, 2016). United States is the leading producer as well as exporter of soybeans with around 90 % of the country's oilseed production coming from this wonderful legume seeds. The farmers of the state of Arkansas has a soybean cultivation on around 3.3 million acres of land in 41 counties out of the total 75, which generates an income of about \$1.7 billion annually. Thus soybeans stand as one of the top three cash crops of Arkansas with farmers (Soybean Production in Arkansas, 2014).

2.1.3 Phenolic Compounds:

Phenolic compounds are classified as simple phenols or polyphenols based on the number of phenol rings in the molecule. Phenol compounds are a class of chemical compounds consisting of a hydroxyl group (—OH) bonded directly to an aromatic q^2 hydrocarbon group (Khoddami *et al.*, 2013). The simplest of the class is phenol, also called as carboic acid ($\text{C}_6\text{H}_5\text{OH}$). Phenolic structures have the possibility to strongly interact with proteins because of

their hydrophobic benzenoid rings and hydrogen-bonding potential of the phenolic hydroxyl groups, which make the phenolics, work as antioxidants. Polyphenols are secondary metabolites of plants which are found in abundance in fruits, vegetables as well as legumes such as soybeans and they impart color, flavor and sensory properties like sweetness, bitterness and astringency (Floridi *et al.*, 2003). They are compounds form the most abundant antioxidants in human diet (Tapiero *et al.*, 2002). Phenolics in plants are involved in providing immunity against ultraviolet radiation, different plant pathogens and parasites. Polyphenols are classified into different categories such as phenolic acids, flavonoids, stilbenes and lignans (Figure 2) depending upon the number of phenol rings that they contain (Pandey and Rizvi, 2009). Many studies have reported the relationship between antimicrobial activity and total or individual phenolics extracted from different sources (Rauha, 2000; Cavanagh, 2003; Puupponen-Pimiä, 2005; Pereira *et al.*, 2006; Ayala-Zavala *et al.*, 2010).

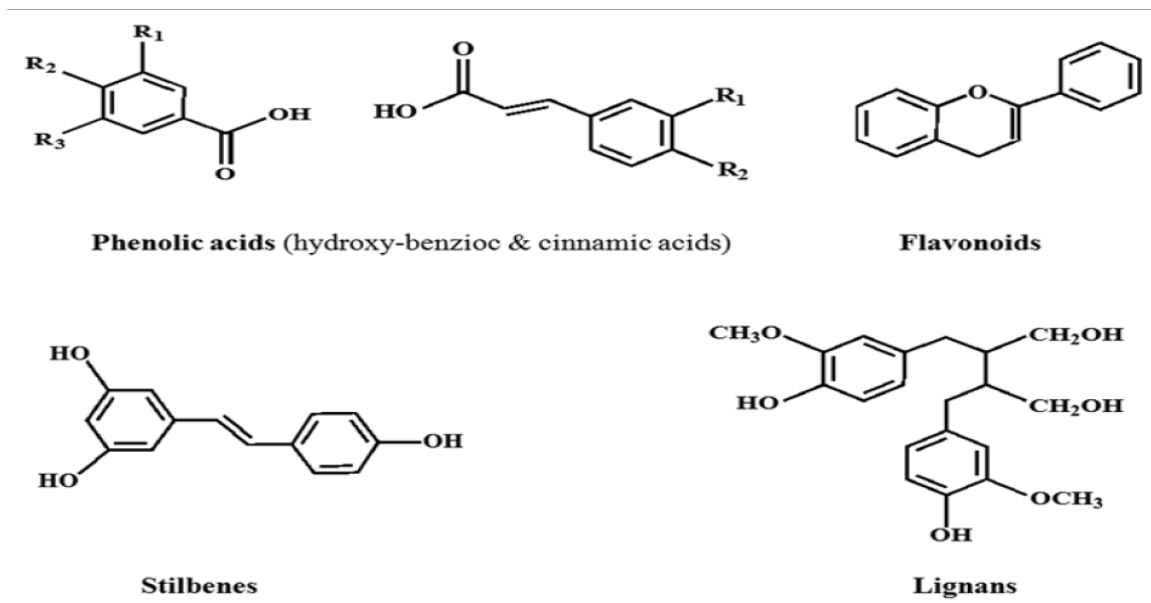


Figure 2. Structures of common phenolic compounds (Pandey *et al.*, 2009)

2.2 Foodborne illness concern on health and economics:

Foodborne illness is a global issue with around 600 million cases of foodborne illness and 420,000 deaths around the world each year due to consumption of food contaminated by various pathogens (Guerra *et al.*, 2016). Centers for Disease Control and Prevention (CDC) reports that every year nearly 1 in 6 or about 48 million people become ill with 128,000 hospitalizations and 3,000 deaths from foodborne illnesses in the United States (CDC, 2015; Kadariya *et al.*, 2014). Illnesses and deaths due to foodborne illness with the source as poultry and poultry products have led to serious concerns over the safety of public health (Wu *et al.*, 2015). Foodborne illnesses are estimated to cause a huge economic loss of around 15.5 billion in the United States alone (Hoffmann *et al.*, 2015). Even though the different United States government agencies have attempted to control the contamination of food products by ensuring strict regulations in food manufacturing facilities, foodborne pathogens have been thriving and are considered as the main causes of foodborne illnesses in the country (Buzby *et al.*, 2001). The public health tracking system for disease outbreaks has recorded that there are about 31 known pathogens which include different types of bacteria, parasites and viruses that cause serious illness in humans (CDC, 2014).

Bacteria are the main reason for many food poisoning cases, generally because of improper food handling. Some bacteria, in small amounts, are not harmful, and they do not affect human health due to the fact that the human body is equipped to fight them. On the other hand, there are different kinds of harmful bacteria that are multiplying and spreading in human and they cause many diseases. Researchers predicted that pathogens that were involved in most foodborne disease (FBD) were *norovirus* (5.5 million, 58%), nontyphoidal *Salmonella*. (1.0 million, 11%), *Clostridium perfringens* (1.0 million, 10%), and *Campylobacter*. (0.8 million, 9%). The

leading causes of FBD in the United States are *Salmonella*, *Escherichia coli* and *Campylobacter* (Scallan *et al.*, 2011& Kadariya *et al.*, 2014).

The infections from these pathogens can occur when food is not cooked very well or not cooled properly (Mead *et al.*, 1999, Crim *et al.*, 2014). Chicken can be infected by *Salmonella*, *Campylobacter* and *Escherichia coli*. Quantitative Risk Assessment (QRA) by FAO /WHO has found that all strains have the same pathogenic potential and there is a growing problem of antimicrobial impedance of diseases associated with chicken (Mbata, 2005).

2.3 Foodborne pathogens associated with Chicken:

Chicken can be contaminated with different pathogens that cause many diseases in humans. This is an important public health issue. Foodborne illness occur on raw chicken, undercooked product during several steps such as manufacture, package, transport the product to the markets due to thousands kinds of pathogens are present in our environment (Bruhn & Schutz, 1999). In the United States several studies reported that harmful pathogens were found on chicken breasts (Zhao *et al.*, 2001). These bacteria were *Salmonella*, *Campylobacter*, verotoxigenic *Escherichia coli*, *Arcobacter* spp, *Helicobacter* spp and *Listeria monocytogenes*. Numerically, the most significant agents are *Salmonalle* and *Campylobacter*. In 2001 in the European Union (EU), there were 157822 person that become ill due to salmonellosis and 156232 illness by *Campylobacter* enteritis and the figures are likely to be considerably higher (Cavitte, 2003). Contaminated raw or undercooked poultry are a significant source of transmitting these food-borne pathogens. Additionally, in food processing environments *Salmonella*, *E. coli* and *Campylobacter* are required to survive a multitude of fatigues which demands that they use specific survival mechanisms. Contact with farm animals and pets as well as person-to-person transmission have also been found as sources of human infections with

Campylobacter, *Salmonella*, and Shiga toxin-producing *Escherichia coli*. Most *E coli* bacteria (STEC) (Tauxe, 1997). Because of their significant risk on raw poultry skin, the Food Safety & Inspection Services (FSIS) of US Department of Agriculture (USDA) have developed compliance guidelines for the control of *Salmonella*, and *Campylobacter* (Wang *et al.*, 2014).

***Salmonella* Typhimurium**

Salmonella is a rod shaped, gram negative, non-spore forming bacteria that is the most frequent cause of foodborne illnesses. It is a bacterium that belongs to the family of enterobacteriaceae. The pathogen causes salmonellosis, which affects the intestinal tract. *Salmonella* bacteria typically lives in animals, specifically, in poultry, swine and human intestines and are shed through feces. The symptoms of this kind of bacteria are diarrhea and abdominal cramps 12 to 72 hours after infection. Each year, there are around 400,000 cases of salmonellosis (Berger *et al.*, 2010). There were one million salmonellosis cases in the United States with 19,000 hospitalizations and 380 deaths (CDC, 2016).

Foodborne illnesses caused by *Salmonella* have been linked to the consumption of tomatoes, melons, sprouts, poultry, peanuts, and other food products. *Salmonella* has been associated with poultry products (Bryan and Doyle 1995; Yang and others 2001). United States Department of Agriculture (USDA) reported that 25% of chicken is contaminated with *Salmonella*, with estimated 1 million cases annually (DeWaal, 1996). CDC and the U.S. Department of Agriculture's Animal and Plant Health Inspection Service (USDA-APHIS) found that eight separate outbreaks of human *Salmonella* contamination are related with chicken (CDC, 2016). Most *Salmonella* found on poultry are non-host specific, which causes human food poisoning. The most common serotypes in the United States are both Typhimurium and Enteritidis (Cormican *et al.*, 2002). Chickens skin are carries of this pathogen and when they

come into the processing plant can cause contamination in the final poultry products (Morris and Wells 1970; Bryan and Doyle 1995; Heyndrickx *et al.*, 2002). Acute fatal and chronic Salmonellosis happens when chicken is contaminated by *Salmonella* serovars (Hofstad *et al.*, 1992& Chappell *et al.*, 2009). Literature information is available on *Salmonella* contamination and the mechanism of transfer to humans (Parker, 1990; Wales& Davies, 2011). *Salmonella* can grow in temperatures ranging from 20-47°C and so can survive very well in chickens as they have a body temperature of 41- 42°C (Troxell *et al.*, 2015; CDC 2016). Several researchers used natural antimicrobial to inhibit *Salmonella* from chicken skin (Goode *et al.*, 2003; Touch *et al.*, 2004).

Campylobacter jejuni

Campylobacter jejuni is a genus of Gram-negative, microaerophilic, oxidase-positive, nonfermentative bacteria. *Campylobacter* species are spiral shaped and has the ability to move via unipolar or bipolar flagella. *Campylobacter jejuni* is considered to be the most common cause of bacterial illness (Hajieh *et al.*, 2016). *Campylobacter jejuni* causes an annual approximate 850,000 cases of disease burden and more than 8000 hospitalizations exist in the United States (Scallan *et al.*, 2011). In 2014 in the United States, the Active surveillance through the Foodborne Diseases Active Surveillance Network (FoodNet) reported that *Campylobacter* causes almost 14 diagnosed every year for each 100,000 persons and 76 persons die due to the infections (CDC, 2014). The symptoms of *Campylobacter jejuni* are diarrhea and bloody diarrhea. There are serious consequences in the long term. Some people may develop Guillain-Barré syndrome which is a rare disease that can lead to paralysis, while other people develop arthritis. The infection of this bacteria is more current in the summer months than in the winter because the temperature has the ability to affect behavioral factors. Also, *Campylobacteriosis*

infection occurs in infants and young adults considerably than other ages and in men more than women (Patrick *et al.*, 2004; CDC, 2014).

Campylobacter jejuni is one of the main source of human pathogenic illness. The reason for the increasing number of human diseases of campylobacteriosis is not known, but poultry is the major source of human contamination (Harrison *et al.*, 2001; Moore *et al.*, 2002; Anonymous, 2003). Because thermophilic *Campylobacters* are mainly *Campylobacter jejuni*, thermophilic *Campylobacters* become main cause of human campylobacteriosis (Dingle *et al.*, 2001). Wilson *et al* (2008) reported that chicken is the main source of campylobacteriosis while wild animal and environmental sources are responsible for only 3% of the infection of the *Campylobacter jejuni*. As a result, health authorities are focusing significantly on reducing the contamination of chicken products by biosecurity measures, which has not been very effective (Humphrey *et al.*, 1993). Many studies indicate that *Campylobacter* are found in chickens as a natural inhabitant in their intestines and that the bacterium can be isolated from the feathers and skin of chickens (Berrang *et al.*, 2000 & Atterbury *et al.*, 2003). Whyte *et al* (2001) studied the addition of hyperchlorite to water in scalding and chilling tanks on the reduction of pathogens such *Campylobacter* present on chicken skin. Despite the use of various techniques to reduce/eliminate *Campylobacter* during the slaughtering and processing of birds, it has been found that fresh poultry harbors *C. jejuni* at levels ranging from 10^2 to 10^5 per carcass and 89% of chicken skin samples were found to harbor the organism (Jacobs-Reitsma, 2000).

***Escherichia coli* O157:H7:**

Escherichia coli O157:H7 is a gram negative, rod-shaped foodborne pathogen. There are 6 different serotypes: enteroaggregative, enteroinvasive, enteropathogenic, enterotoxigenic, diffuse adherent, and enterohemorrhagic. In 1982, *Escherichia coli* was discovered in

hamburgers from fast food chains (Tauxe 1997; Feng 2012). *Escherichia coli* (STEC) cause approximately 100,000 illnesses, 3,000 hospitalizations, and 90 deaths annually in the United States (Mead *et al.*, 1999; CDC, 2009). In 2005, a study reported estimated the annual cost of *E. coli* O157:H7 illnesses to be \$405 million, which included \$370 million for premature deaths, \$30 million for medical care, and \$5 million for lost productivity (Frenzen *et al.*, 2005).

Escherichia coli can be found naturally in the human gut, which can be beneficial to the host to inhibit the harmful pathogens colonization in the gut. On other hand, *Escherichia coli* can be harmful due to the fact that it has the ability to produce Shiga toxins, so it causes disease for human (Feng, 2012). Infection of the *E coli* O157:H7 causes hemorrhagic colitis (HC) and the symptoms of it are severe abdominal cramp and bloody diarrhea. Also, it can cause dehydration and kidney failure, which is known as Hemolytic-Uremia Syndrome and is potentially life-threatening disease. *Escherichia coli* has the high risk for young children and the elderly while healthy adults recover within 5-10 days (Food safety, 2013).

Escherichia coli has always been associated with outbreaks in which the food source is raw meat or meat products. *E coli* O157:H7 infections secondary to respiratory infections in broilers, egg-type pullets and layers, and turkeys, and this is an important disease scenario. *E coli* contamination comes during slaughter and when infected chicken intestines or feces come in contact with the other chicken (Silagyi *et al.*, 2009). Moreover, the ability of *E coli* O157:H7 to compose a biofilm on chicken surfaces (skin) creates a high risk of cross contamination (Jackson, 2007).

2.4 Heart Disease Facts:

Heart disease is a range of conditions that affects the human heart, which can cause death in the end. There are many different types of heart diseases such as hypertension, coronary heart

disease, heart attack, congestive heart failure, and congenital heart disease. High blood pressure (Hypertension) occurs if the systolic blood pressure is increased more than 140 mm Hg or the diastolic blood pressure is increased more than 90 mmHg (Chobanian, 2008). Hypertension is one of the major causes of heart disease throughout the world and is estimated to be the cause of death in about 375,000 Americans each year (American Heart Association, 2015). The national Health and Nutrition Examination reported that between 2005–2008 there are 76,400,000 or approximately 33.5%, men and women in the US that have hypertension and 44% of African Americans have hypertension, which is the highest percentage of that disease around the worlds (Roger *et al.*, 2011). The world Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) indicate that the primary cause of death in several countries, such as the UK, USA, Canada and Australia, is heart disease. Additionally, 1 in every 4, or about 610,000 people die every year in the USA because of heart disease (CDC, 2015). Annually, in the US about 735,000 people are infected by heart attacks. 525,000 of those people have a first heart attack while 210,000 occurs in humans who have a heart attack in the past (Mozaffarian *et al.*, 2011; CDC, 2015). In the United States alone annually the health care services for heart diseases are estimated to cost approximately \$ 207 billion (Mozzafarian *et al.*, 2016).

There are many reasons which causes heart diseases such as high blood pressure, diabetes, obesity, high cholesterol, excessive alcohol use and smoking. Almost 47% of Americans have one or two of these three danger factors (Fryar *et al.*, 2012 & American Heart Association 2014). High blood pressure is one of the risk factors of heart failure which causes left ventricular hypertrophy and cause less active muscle relaxation between heart beats. There are many different symptoms for heart failure such as shortness of breath, swelling in the feet, ankles, difficulty sleeping flat in bed, bloating, irregular pulse, nausea, fatigue and greater need

to urinate at night (Malik *et al.*, 2014). For treatment of the hypertensive heart disease it should assist in decreasing high blood pressure and inhibits the production of angiotensin II (Smith *et al.*, 2015).

2.5 Angiotensin- converting enzyme (ACE) inhibitory:

Angiotensin- converting enzyme (ACE) works significantly in circulatory homeostasis. It is found in plasma and its membrane restricted enzyme in endothelial cells and epithelial cells. ACE stimulates the conversion of angiotensin I to the potent vasoconstrictor octapeptide angiotensin II and activates the vasodilator bradykinin (Shi *et al.*, 2010). Angiotensin I can be formed by angiotensinogen with renin-angiotensin system (RAS), which is produced from the liver and progressive in the blood. Angiotensin I can be turns into Angiotensin II by the enzyme angiotensin converting enzyme (ACE). Thus, the human body can produce Angiotensin II in the blood, which has the ability to contract the muscles surrounding blood vessels, so narrowing the vessels, which causes high blood pressure (hypertension) (Azizi & Ménard, 2004). Additionally, angiotensin II increases the size of cardiovascular structures, which become thicker and stiffer (hypertrophy). This leads to cholesterol deposits and blockages in the arteries, which make the heart attacks and strokes, happen (Sweitzer, 2003).

The angiotensin-I-converting enzyme (ACE) inhibitor is physiologically significant in blood pressure regulation. It assists to treat the increase of blood pressure (Hong *et al.*, 2008). ACE inhibitors can decrease or prevent activity of the enzyme ACE, so it inhibits producing angiotensin II (Figure 3). Therefore, blood pressure decreases, blood vessels are relaxed and the blood volume reduces. The reducing of blood pressure assists the heart to pump blood easily and it reduces the chances of heart failure. This is the reason ACE inhibitors were initially used to lower blood pressure in humans with hypertension (Farquharson & Struthers, 2000; Granger *et*

al., 2003). Sweitzer (2003) reported that using ACE inhibitors appears to be a particularly influential treatment for decreasing heart attacks in patients. There are many ACE inhibitors such as Captopril, Lisinopril, and Enalapril that are used for hypertension treatment, which assist to reduce blood pressure (Hong *et al.*, 2008; Hernández-Ledesma *et al.*, 2011).

Even though those synthetic compounds have the beneficial effect of inhibiting hypertension, they have side effects such as coughing, taste disturbances, and skin rashes. Therefore, food derived ACE inhibitors are preferred as an alternative which can prevent hypertension without harmful side effects at a cheap cost (Hong *et al.*, 2008; García *et al.*, 2013). Soybean is one of the plant sources that are producing ACE inhibitors with a low price (Segura-Campos *et al.*, 2013). There are many compounds derived from soybeans, such as globulin or glycinin, that are reported to be robust competitive inhibitors of ACE and to be resistant to digestion by proteases of the gastrointestinal tract (Mallikarjun Gouda *et al.*, 2006). Kwon *et al.* (2008) concluded that phenolic-enriched, which is extracted from eggplant, had high α -glucosidase inhibitory activity, which works as an angiotensin I-converting enzyme (ACE) inhibitory activity. A study reported that extracted phenolics from two of the most popular species of edible bamboo shoots in Korea (*Phyllostachys pubescens* and *Phyllostachys nigra*) have high angiotensin converting enzyme (ACE) inhibition activity (Park & Jhon, 2010).

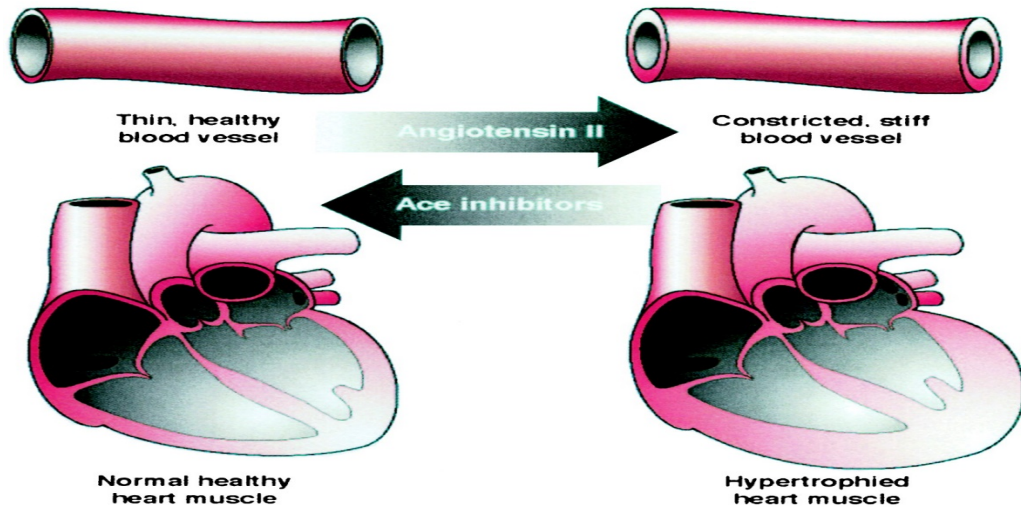


Figure 3. Showing the impacts of angiotensin II on the blood vessels and heart (Sweitzer, 2003)

Chapter 3: Preparation of phenolic extracts from selected colored soybean varieties and quantify total phenolics.

3.1 Introduction

Soybeans rank the second among the most planted field crops after corn in the United States with the state of Arkansas growing about 3.3 million acres of soybeans annually (Soybean Production in Arkansas, 2014). The seed coat of soybeans known as soybean hull is a by-product of the soybean oil and soybean meal industry constitute about 10% of the whole seed (Duenas *et al.*, 2006). Soybean hulls are a rich source of dietary fiber comprising of 86% polysaccharides such as pectin, cellulose and hemicellulose along with polyphenols, phytic acid, vitamins and minerals (Liu *et al.*, 2013; O'Bryan *et al.*, 2014). Polyphenols are secondary metabolites of plants which are found in abundance in fruits, vegetables as well as legumes such as soybeans and they impart color, flavor and sensory properties like sweetness, bitterness and astringency (Floridi *et al.*, 2003). They are compounds possessing one or more hydroxyl groups attached to one or more aromatic hydrocarbon groups and form the most abundant antioxidants in human diet (Tapiero *et al.*, 2002).

Soybean hulls come in a range of colors like yellow, green, blue, brown and black or might have a mottled appearance. The highest concentration of the polyphenolic compounds like anthocyanins and proanthocyanidins present in soybean hulls especially those with dark colored seed hulls, and are, responsible for giving them a variety of colors (Todd & Vodkin, 1993; Duenas *et al.*, 2006; Xu & Chang 2008; Malenčić *et al.*, 2012).

Phenolic compounds or phenolic phytochemicals (phenolic acids, flavones, flavonols, flavanones, flavanone, isoflavones, and anthocyanidins) are vastly found in fruits, vegetables and Legumes such as soybean hulls. Phenolics in plants are involved in immune protection and

defense against ultraviolet light (D'Archivio *et al.*, 2007). Polyphenolics can be extracted from plant materials using different solvents such as ethanol, methanol, acetone, ethyl acetone or their combinations with different proportions of water. But for extracting phenolics from anthocyanin rich plant sources commonly acidified organic solvents such as ethanol or methanol is used (Dai & Mumper, 2010). Methanol can be used to extract free and simple phenolics for analytical purposes, but it is not acceptable as a food grade solvent since it is considered as an environmental hazard due to its toxicity (Shi *et al.*, 2005). Extraction of phenolics using ethanol is generally recognized as safe (GRAS) for human consumption (Li *et al.*, 2014).

The total phenolics can be calculated by measuring the absorbance of the extract and comparing these with that of the standard curve of a phenolic compound. Kähkönen *et al* reported polyphenols in 26 different kinds of berries including biphenyls, flavonoids, phenolic acids, and other simple phenolics, such as caffeic, chlorogenic, ferulic, sinapic, and p- coumaric acids. The main constituents of phenolic composition found in the plants, are Flavonoids (flavonols, anthocyanins [ACY], proanthocyanins, and catechins) and phenolic acids. Malenčić *et al* (2012) indicated that varieties of soybean have different concentrations of polyphenols. They observed that yellow seed hull has the highest amount of total isoflavones, especially genistein, while black, brown, and other seed hulls contained more of anthocyanins.

3.2 Materials and methods:

Materials

Soybean hulls with four different colors were used for the present study, which were yellow (R08- 4004), dark brown (R09- 349), brown (R07-589), and black (R07-1927). All the four types of soybeans with different seed hull colors were supplied by Dr. Pengyin Chen, Professor in the Department of Crop, Soil, and Environmental Sciences at the University of

Arkansas. Methanol, Folin–Ciocalteu reagent and sodium carbonate were purchased from VWR International, Inc (Suwanee, GA, USA) and Sigma-Aldrich, Inc.). Chlorogenic acid was purchased from Aldrich Chemical Co. (Milwaukee, Wis., U.S.A.).

Preparation of uniform size fine particles from soybean hulls

Soybean seeds from each variety were dehulled using a dehuller (Yamamoto FC2K instrument, Siba International Corp, Tokyo, Japan). The hulls were ground using a sample grinder (IKAWERKE grinder model M20, Ika Works, Inc., Wilmington, NC, U.S.A.), passed through an 80-mesh sieve (W.S. Tyler Inc., Mentor, OH, U.S.A.) to obtain uniform particles. The powder thus obtained from the four different colored soybean hulls were stored in air-tight plastic bags at 4°C before analysis.

Extraction of Total Phenolics

The phenolic compounds were extracted using aqueous methanol (v/ v), which had been proven to be the most efficient solvent for determine and quantify the total phenolics (Chidambara Murthy *et al.*, 2002). However, methanol extraction was done only for quantification of the total phenolics in the hull extract, as methanol is considered to be toxic and thus an environmental hazard. For all other experiments ethanol was used to extract phenolics from soybean hull powder. The extraction of total phenolics from soybean seed hulls was done by following the method as described by Khanal *et al.*, 2009, with some modifications. A mixture was prepared with 25 ml of 70% aqueous methanol/ ethanol and 5g of the seed hull powder, which was stirred for 10 min at ambient temperature and then sonicated for 10 min. The mixture was vacuum filtrated to separate the phenolic extract from residue. This extraction procedure was repeated twice to extract the residual phenolics.

Total phenolics determination:

The total phenolic content of the soybean hull extracts obtained from the four different colored seed hulls using methanol was determined using the Folin–Ciocalteu method (Singleton & Rossi, 1965). Ten mg of each phenolic extract was weighed into test tube and vortexed with 50 mL deionized (DI) water. One mL of the mixture thus obtained was taken in another test tube to which 7 mL of DI water, 1 mL of 0.25N Folin–Ciocalteu reagent, and 1mL of 1N sodium carbonate were added. The test tubes were then vortexed, covered by aluminum foil and incubated for 2 hours at ambient temperature. The A spectrophotometer (Shimadzu Model UV-1601, Kyoto, Japan) at 726nm was used to measure the absorption of the solutions. The milligrams of chlorogenic acid equivalent (CAE) per gram dry weight (DW) of soybean seed hulls (mg CAE/g DW) was calculated using the following formula: Total phenolics (mg CAE/g DW) = $(101.8 \times A - 15.1) \times 5$; where A is the absorbance at 726 nm. The experiment was replicated three times and the total phenolic content was calculated using a standard curve obtained with the chlorogenic acid standard.

Statistical analysis

All values are reported as means of triplicate samples from each variety of soybean hulls to compare the amount of total phenolic. The JMP 10.0 software (SAS Institute, Cary, NC) was used to for one-way analysis of variance to determine the highest amount of the total phenolics. Analysis of variance (ANOVA) was performed and significant difference was determined at $P < 0.0001$.

3.3 Result and discussion:

Total phenolic contents of soybean hulls:

Total phenolic contents determined using Folin–Ciocalteu method and expressed as milligrams of chlorogenic acid equivalent (CAE) per gram dry weight (DW) of soybean seed hulls (mg CAE/g DW) for the four different colored soybean hulls are given in Table 1. The phenolic contents of the four different colored (black, brown, dark brown and yellow) soybean hulls ranged from 1.63 to 4.29 mg of CAE/g DW, with the yellow soybean hull having the lowest concentration of phenolics. The highest total phenolic content (4.29 mg CAE/g DW) was obtained for the hull extract of the soybean variety R07-1927 (black), which is the darkest colored soybean, which was not significantly different ($P = 0.0099$) from the phenolic content of the dark brown soybean hull (3.85 mg CAE/g DW). The total phenolic contents of the black and dark brown varieties were found to be significantly different ($P < 0.05$) from the brown and yellow colored seed coats which had phenolic concentrations of 3.31 mg CAE/g DW and 1.63 mg CAE/g DW respectively.

The differences in the total phenolic content of the four varieties of soybean hulls used in the present study points out that the color of soybean hulls has an influence on the total phenolics and perhaps of the individual phenolics extracted. Highest concentrations of phenolics was obtained for the hull extracts from black and brown soybeans which are dark colored. Our results are in concurrence with the results of the study by Malenčić *et al.*, 2012, which showed that the black and brown colored seeds had highest concentrations of polyphenols. The presence of high concentrations of phenolics, such as anthocyanins and proanthocyanidins, in the seed coats of black soybean, are major determinants for the color (Todd & Vodkin, 1993; Astadi *et al.*, 2009). We also observed that the lowest concentration of phenolics among the varieties of soybeans

tested was obtained for the yellow colored hull. Hence it might be inferred that the darker colors of soybean coats are due to the presence of higher concentrations and types of phenolics.

3.4 Conclusion

The present study showed differences in total phenolic contents of soybean varieties with different colored seed coats such as yellow (R08- 4004), dark brown (R09-349) brown (R07-589) and black (R07-1927). The highest total phenolic content (4.29 mg CAE/g DW) was obtained for the hull extract of the soybean variety R07-1927 (black) while the lowest total phenolic content (1.63 mg CAE/g DW) was obtained for the conventional soybean varieties R08-4004 (yellow). This observation can be due to differences in varieties.

Table 1. Total phenolic contents in seed coat powder from four different varieties of soybean

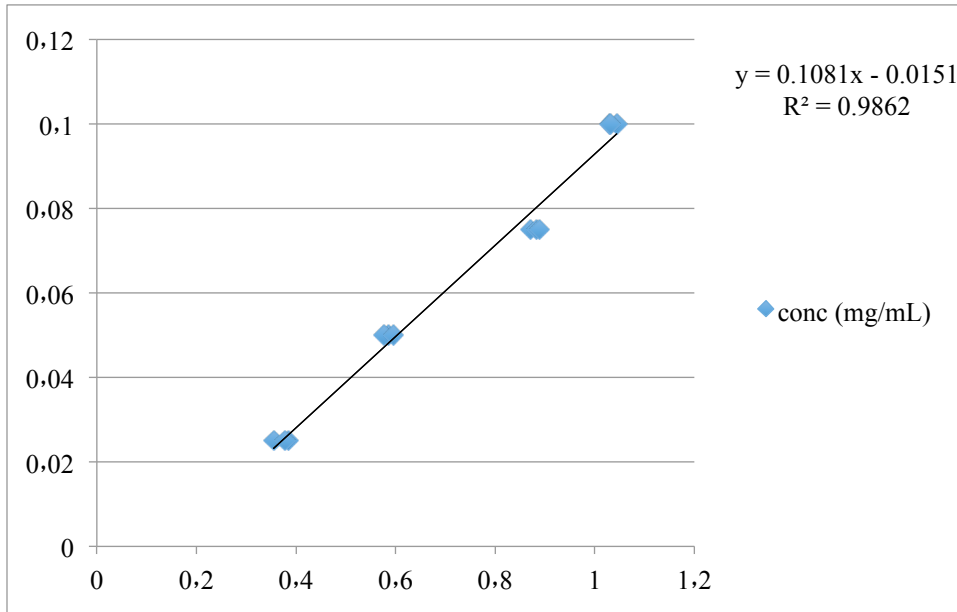
Soybean variety	Hull color	**Total phenolics (mg CAE/g DW)
R07-1927	Black	4.29±0.12 ^a
R09-349	Dark brown	3.85±0.50 ^a
R07-589	Brown	3.31±0.16 ^b
R08-4004*	Yellow	1.63±0.05 ^c

* Conventional soybean variety

Values are means ± standard deviation of three determinations.

**Values in the same column with the same letter are not significantly different (P <.0001).

Figure 4. Chlorogenic Acid standard curve for the determination of phenolic acids concentration in the samples



$$Y = 0.1081x - 0.0151$$

Y = phenolic acid concentration in mg CA equivalents/ g DW

X = absorption readings

Chapter 4: Determination of the minimum inhibitory effects of the extracts on *S. Typhimurium*, *E coli* 0157:H7, and *Campylobacter jejuni* in broth cultures

4.1 Introduction

Foodborne illness or foodborne infection is a common yet serious public health problem all over the world. Bacteria such as *Salmonella*, *Campylobacter*, *E coli*, *Listeria*, *Clostridium* and *Staphylococcus* are some of the most common causes of foodborne infections. Despite methods to reduce or eliminate bacterial foodborne pathogens, foodborne illnesses still continue to pose a major threat to public health (CDC, 2016). The control of pathogens may safely decrease the foodborne disease outbreaks (Kiran *et al.*, 2008). There are more than 80,000 chemical preservatives that are used to inhibit the growth of pathogenic bacteria and some of them are used to protect the quality of chicken meat. Natural antimicrobials have started slowly replacing addition of traditional antimicrobials such as different antibiotics to food and food products due to increasing consumer awareness (Cetin-Karaca & Newman, 2015). So there is a need to determine the minimum inhibitory concentrations (MIC) of new and novel plant derived phenolics (Phanthong *et al.*, 2013). In recent years, phenolic compounds have received attention because of their significant functions as antioxidant and antimicrobial (Liu, 2003). Because of their antibacterial, antifungal and antiviral activity, and antioxidant properties, phenolic compounds have been researched for many years (Hulin *et al.*, 1998; Suppakul and others 2003; Lai and Roy 2004; Cushnie and Lamb 2005; Fattouch *et al.*, 2007; Szabo and *et al.*, 2010). Phenolic extracts and individual phenolics separated from plants have been used as antimicrobials to inhibit *Salmonella*, *E coli*, and *Campylobacter* (USDA, 2011). They are compounds possessing one or more hydroxyl groups attached to one or more aromatic hydrocarbon groups and form the most abundant antioxidants in human diet (Tapiero *et al.*,

2002). Phenolics in plants are involved in providing immunity against ultraviolet radiation, different plant pathogens and parasites (Pandey and Rizvi, 2009). The objective for the present study was to determine the inhibitory effect of total phenolic extracts from hulls of selected colored soybean varieties on the inhibition of the pathogens such as *Salmonella* Typhimurium, *E. coli*, and *Campylobacter jejuni* in cultures.

4.2 Materials and methods:

Materials

Soybean hulls with four different colors were used for the present study, which were: yellow (R08- 4004), dark brown (R09- 349), brown (R07-589), and black (R07-1927). All the four types of soybeans with different seed coat colors were supplied by Dr. Pengyin Chen, Professor in the Department of Crop, Soil, and Environmental Sciences at the University of Arkansas. Agar slant cultures of *E. coli* O157:H7 (GFP-labeled ED 14) and *Salmonella* Typhimurium (ATCC 14028) were provided by the Center for Food Safety Research Laboratory, University of Arkansas. *Campylobacter jejuni* NCTC 11168 was provided by Dr. Kwon, Department of Poultry Science, University of Arkansas. Agar media for the determination of minimum inhibitory effects were purchased from Difco™ (a division of Becton, Dickinson and Co., USA). All the other chemicals used were reagent grade and purchased from VWR International, Inc. (Suwanee, GA, USA), Sigma-Aldrich, Inc, USA and Aldrich Chemical Co. (Milwaukee, WI, USA).

Extraction of Phenolics:

The phenolic compounds were extracted using aqueous ethanol as described in chapter 3.

Preparation of the Cultures:

Ten μl of frozen stock cultures of *Salmonella* Typhimurium, *Escherichia coli* O157:H7 and *Campylobacter jejuni* were used to inoculate in 10 mL of brain heart infusion homogenate (BHI) broth for *Salmonella* Typhimurium, *Escherichia coli*, and Mueller Hinton broth (MH) for *Campylobacter jejuni* at 200 rpm agitating incubator (Edison NJ, U.S.A.) Cultures of *Salmonella* and *E coli* were incubated at 37°C for 24 h, while *C. jejuni* cultures were incubated for 48 h in a micro-aerobic atmosphere consisting of 85% nitrogen, 10% carbon dioxide, and 5% oxygen because it killed by oxygen, so it needs less oxygen than the amount in the atmosphere. After completion of incubation, the cultures (10 μl) from the first passage were passed into 10 mL of fresh respective broth. The cultures were incubated under conditions similar to the first passage to make sure that the culture of the bacterial was in the exponential growth phase. Cultures from second day were used for all experiments for (10^9 log CFU/mL) which is the high level that can offer a noticeable decline in the status of inhibitory action. (10^9 log CFU/mL) was diluted to (10^4 log CFU/mL) which is the same range of bacteria in the food.

Determination of inhibitory effects of Soybean hull extracts on bacteria in cultures:

Frozen strains of *Salmonella* Typhimurium, *Escherichia coli*, and *Campylobacter jejuni* stored at -70°C were passed twice in respective broth media to revive the bacteria. For each passage, Brain Heart Infusion (BHI) broth was used to culture *S. Typhimurium* and *E. coli* O157:H7 and the cultures were incubated for 24 h to obtain good growth. *C. jejuni* was cultured in Mueller Hinton (MH) broth and incubated for 48 h in a micro-aerobic atmosphere consisting of 85% nitrogen, 10% carbon dioxide, and 5% oxygen. Cultures after the second passage were used at a concentration of 10^4 and 10^9 CFU / ml for the experiments. 100 μL of phenolic extracts from the soybean hulls in different concentrations (0.5%, 1%, 2%, 6%, and

10%) was added to 100µL of bacterial culture (*S. Typhimurium*, *E. coli* and *C. jejuni*) in screw capped vials and incubated for 3 different time periods (1day, 3days and 6days). From the incubated mixtures 20µL was added to 180µL of sterilized phosphate buffer saline (PBS, 20 mM) in a 96-well microtiter plate, serially diluted and plated on to selective agar plates (XLT4 agar for *S. Typhimurium*; MacConkey-Sorbitol agar for *E. coli* and Mueller Hinton agar for *C. jejuni*) in triplicate. All the plates for enumeration of *S. Typhimurium* and *E. coli* were incubated at 37°C for 24h (Edison, N.J., U.S.A.), while the plates for *C. jejuni* were incubated at 42°C for 48h.

Statistical analysis

All the experiments were replicated three times and the values were calculated as means of the three determinations. JMP 10.0 software (SAS Institute, Cary, NC) was used to perform one way analysis of variance on the data. Analysis of variance (ANOVA) was used to determine the minimum inhibitory concentrations of the soybean hull extracts in cultures and significant difference was determined at $P < 0.05$.

4.3 Results and Discussion

The antimicrobial activity of the ethanolic total phenolic extracts from four different colored soybean seed hulls (R08-4004, R09-349, R07-589 and R07-1927) were investigated on foodborne pathogens such as *Salmonella Typhimurium*, *Escherichia coli*, and *Campylobacter jejuni* in broth cultures. The antimicrobial activity of total phenolic extracts from each variety of soybean hull was studied at five different concentrations of 0.5%, 1%, 2%, 6%, and 10% on two levels of each type of bacterial cultures (a high level of 10^9 CFU/ ml and low level of 10^4 CFU/ ml). All the cultures were incubated for three different time periods of 1, 3 and 6 days and their antimicrobial effects are shown in Tables 2- 17.

Table 2 shows the log reductions of *Salmonella* Typhimurium incubated with phenolic extracts of soybean hulls for 1 day from an initial level of 10^9 CFU/mL. Overall, the highest log reduction of 0.80 was observed for the R07-1927 (black soybean hull) with a 10% concentration of the extract from bacterial levels of 10^9 CFU/mL compared to other varieties. The same concentration of yellow soybean variety, R08-4004 produced only limited inhibitory action with 0.37 log reduction after incubation for 1 day. But the log reductions with the dark brown and brown colored hull extracts at 10 % concentration were almost the same. All the four hull extracts at concentrations of 0.5%, 1% and 2% did not show any significant differences in log reductions of *S. Typhimurium*. Table 3 shows the log reduction of *Salmonella* Typhimurium incubated with phenolic extract for one day with a low level of bacteria at 10^4 CFU/mL. When bacterial levels became low, higher log reductions were observed, with the R07-1927 extract producing the highest log reduction of 0.97 after day 1. In higher bacterial concentrations, the concentration of autoinducers produced due to quorum sensing, might be reaching levels at which the bacteria are able to defend the actions of the phenolic extracts (Deep *et al.*, 2011). Here also we observed similar trend of log reductions for the other soybean hulls with the yellow variety producing the lowest log reduction of 0.67. No significant difference was observed between the different concentrations of 0.5%, 1% and 2% for all the extracts.

Bacterial counts were also determined after longer incubation time of 3 and 6 days. On incubating for 3 days with the extract of R07-1927 *S. Typhimurium* counts were found to be lowered by 0.93 and 1.20 logs from initial bacterial levels of 10^9 and 10^4 CFU/mL respectively. The lowest log reductions were still seen with the yellow variety extract with 0.67 and 0.77 logs for bacterial levels of 10^9 and 10^4 CFU/mL respectively (Table 4 & 5). Incubating bacterial cultures with the phenolic extracts for a longer period of 6 days also produced similar results as

with incubating for 3 days (Tables 6 & 7). The maximum log reduction of 2.0 logs was observed when *Salmonella* Typhimurium was incubated for 6 days with the most effective extract from the hull of R07-1927.

The phenolic extracts of the 4 different soybean hulls showed a similar type of response with *E coli* O157:H7 as with *S. Typhimurium* when incubated for 1 day (Tables 8 & 9), 3 days (Tables 10 & 11) and 6 days (Table 12 & 13). The 10% concentration of the black variety (R07-1927) produced the maximum log reductions of 0.90, 1.97 and 2.67 for days 1, 3 and 6 respectively, from an initial bacterial level of 10^9 CFU/mL. Similarly from a bacterial level of 10^4 CFU/mL log reductions of 1.20, 2.20 and 3.30 were observed after incubation for 1,3, and 6 days for the above extract. Here also the yellow soybean hull extract was found to have the least inhibitory effects on *E coli* O157:H7 in cultures.

With *Campylobacter jejuni* the soybean hull phenolic extracts produced similar pattern of reductions with incubation periods of 1 day (Tables 14 & 15) and 3 days (Tables 16 & 17). One log reductions were observed after day 1, with the 10% concentration of R07-1927 extract for both the initial bacterial levels of 10^9 and 10^4 CFU/mL. Upon incubating the bacterial cultures with the extracts for 3 days, 2 log reductions were observed for both bacterial levels. The phenolic extract of yellow soybean produced 0.60 - 0.93 and 1.13-1.37 log reductions for days 1 and 3 with the two different levels of bacteria. No colonies of *C. jejuni* could be detected even in the controls after 6 days incubation, as this bacterium is very sensitive to environmental conditions (Park, 2002). Another study reported that *Campylobacter jejuni* survived for only a few days during incubation and does not have the ability to survive environmental stress (Buswell *et al.*, 1998). *Campylobacter jejuni* has poor ability of storing energy to assist growth

due to the fact that *Campylobacter jejuni* does not have 6-phosphofructokinase which is special enzyme for energy metabolism (Velayudhan & Kelly 2002; Stahl *et al.*, 2012).

The log reductions for all the phenolic extracts were found to be higher with the initial bacterial concentration of 10^4 CFU / ml than for 10^9 CFU / ml. Our results show a significant difference in the inhibitory actions of the black and yellow colored varieties with all the three bacterial species, but not much difference was observed between the black, dark brown and brown varieties. Colored soybean seed coats have been shown to possess phenolic acid compounds such as anthocyanins and proanthocyanidins in abundance, but these are not found in the yellow variety (Todd & Vodkin, 1993). The yellow variety however, has more of isoflavones such as genistein and daidzein (Zilic *et al.*, 2013). The difference in the log reductions between the colored soybean hulls and the yellow variety could be due to the difference in the composition of individual phenolic compounds present in the hull extracts.

Research on the inhibition of pathogens by extracts from soybean hulls is limited. Phenolic compounds have been found to have an adverse effect on bacterial growth by inhibiting their metabolic functions (Cushnie & Lamb, 2005). The results of the present study revealed that even though soybean extracts were able to reduce bacterial growth, the antimicrobial activities of these extracts. Study by Park *et al.*, (2011) found variations in the sensitivity of *Salmonella* and *Listeria* when treated with blueberry and muscadine total phenolic extracts. This difference in reaction between the bacteria to the total phenolic extracts might be due to the difference in their cell structures and characteristics (Puupponen Pimiä *et al.*, 2001; Park *et al.*, 2011). Research on inhibition of different foodborne pathogens tested by phenolic extracts from defatted soybean flour, found that gram-positive bacteria showed more sensitivity to the extracts than the gram negative (Villalobos *et al.*, 2015). Gram negative bacteria are more resistant to different types of

antibacterials due to the presence of an outer membrane with lipopolysaccharides which is absent in gram positive bacteria. Even though all the three bacteria in this study are gram negative, their cell walls differ due to the difference in outer membrane proteins and enzymes which lead to difference in permeability to the soybean hull extracts (Winfield and Groisman, 2004, Hobb *et al.*, 2009, Silhavy *et al.*, 2010). It is also possible that there might be some compounds in the total phenolic extracts, which may be interfering with the action of the phenolic compounds. Plant derived individual phenolic compounds were found to inhibit various species of *Bacillus*, *Listeria* and *Clostridium* at concentrations of 5-20 ppm (Cetin-Karaca & Newman, 2015). Total phenolic extracts from different plant sources could have different types of influence on the growth of the same bacteria. Phenolic extracts from berries such as cloudberry, raspberry and strawberry were found to strongly inhibit *Salmonella*, while the extracts from Sea buckthorn berry and blackcurrant were found to have the least effect against *Salmonella* and *E. coli* (Puupponen Pimiä *et al.*, 2001). Very limited information is available on the inhibitory activity of different colored soybean hull phenolic extracts against different foodborne pathogens in the literature.

4.4 Conclusion

The present study showed differences in inhibitory effects of the phenolic extracts obtained from hulls of soybean with different colored seed coats such as yellow (R08-4004), dark brown (R09-349) brown (R07-589) and black (R07-1927). The phenolic extracts at different concentrations were found to inhibit bacteria in cultures producing 1-3 log reductions which was found to vary with the type of bacteria. Maximum log reductions were observed when bacteria in cultures were incubated for longer time periods of 3 and 6 days.

Table 2. The log reductions of *S. Typhimurium* (from 10^9 log) when incubated with phenolic extract for 1 day

Soybean varieties					
Extract concentration (%)	R07-1927	R09-349	R07-589	R08-4004	<i>P</i> value
0.5	0.47±0.05 ^{cA}	0.33±0.05 ^{cB}	0.33±0.05 ^{cB}	0.03±0.05 ^{bC}	<0.0001
1	0.47±0.09 ^{cA}	0.43±0.09 ^{bcA}	0.43±0.09 ^{bcA}	0.07±0.05 ^{bB}	<0.0045
2	0.53±0.05 ^{bcA}	0.43±0.12 ^{bA}	0.47±0.05 ^{bA}	0.10±0.08 ^{bB}	<0.0003
6	0.63±0.05 ^{bA}	0.53±0.05 ^{abA}	0.53±0.05 ^{bA}	0.27±0.05 ^{aB}	<0.0003
10	0.80±0.00 ^{aA}	0.63±0.05 ^{aB}	0.67±0.05 ^{aB}	0.37±0.05 ^{aC}	<0.0001
<i>P</i> value	0.0006	0.0057	0.0028	0.0005	

Values are means ± standard deviation of three determinations; mean values with different lowercase letters in the same column and different uppercase letters in the same row are significantly different ($P < 0.05$).

Table 3. The log reductions of *S. Typhimurium* (from 10^4 log) when incubated with phenolic extract for 1 day

Soybean varieties					
Extract concentration (%)	R07-1927	R09-349	R07-589	R08-4004	<i>P</i> value
0.5	0.67±0.05 ^{cA}	0.60±0.00 ^{cA}	0.53±0.09 ^{bA}	0.07±0.09 ^{cB}	<0.0001
1	0.67±0.05 ^{cA}	0.63±0.00 ^{cA}	0.60±1.14 ^{bA}	0.23±1.17 ^{bcB}	0.0114
2	0.70±0.00 ^{cA}	0.70±0.05 ^{bcA}	0.67±0.05 ^{abA}	0.37±0.05 ^{abB}	<0.0001
6	0.83±0.05 ^{bA}	0.77±0.09 ^{abA}	0.70±0.00 ^{abA}	0.53±0.09 ^{aB}	0.0146
10	0.97±0.05 ^{aA}	0.83±0.05 ^{aB}	0.83±0.05 ^{aB}	0.67±0.05 ^{aC}	0.0017
<i>P</i> value	<0.0001	0.0064	0.0386	0.0034	

Values are means ± standard deviation of three determinations; mean values with different lowercase letters in the same column and different uppercase letters in the same row are significantly different ($P < 0.05$).

Table 4. The log reductions of *S. Typhimurium* (from 10^9 log) when incubated with phenolic extract for 3 days

Soybean varieties					
Extract concentration (%)	R07-1927	R09-349	R07-589	R08-4004	<i>P</i> value
0.5	0.63±0.05 ^{cA}	0.57±0.12 ^{cA}	0.53±0.05 ^{cA}	0.13±0.05 ^{bB}	<0.0006
1	0.67±0.05 ^{cA}	0.65±0.08 ^{cA}	0.57±0.12 ^{cA}	0.13±0.12 ^{bB}	0.0027
2	0.67±0.07 ^{cA}	0.70±0.08 ^{bcA}	0.60±0.08 ^{bcA}	0.23±0.12 ^{bB}	<0.0001
6	0.80±0.00 ^{bA}	0.80±0.00 ^{abA}	0.77±0.05 ^{abA}	0.57±0.05 ^{aB}	0.0003
10	0.93±0.05 ^{aA}	0.93±0.05 ^{aA}	0.87±0.05 ^{aA}	0.67±0.05 ^{aB}	0.0013
<i>P</i> value	0.0002	0.0052	0.0056	0.0002	

Values are means ± standard deviation of three determinations; mean values with different lowercase letters in the same column and different uppercase letters in the same row are significantly different ($P < 0.05$).

Table 5. The log reductions of *S. Typhimurium* (from 10^4 log) when incubated with phenolic extract for 3 days

Soybean varieties					
Extract concentration (%)	R07-1927	R09-349	R07-589	R08-4004	<i>P</i> value
0.5	0.70±0.08 ^{dA}	0.67±0.12 ^{cA}	0.66±0.12 ^{bA}	0.30±0.08 ^{bB}	0.0156
1	0.77±0.09 ^{cdA}	0.73±0.12 ^{bcA}	0.73±0.12 ^{bA}	0.30±0.08 ^{bB}	0.0072
2	0.90±0.00 ^{bcA}	0.73±0.07 ^{bcAB}	0.77±0.05 ^{bB}	0.47±0.12 ^{bC}	0.0018
6	0.97±0.05 ^{bA}	0.93±0.05 ^{abA}	0.83±0.05 ^{bA}	0.73±0.05 ^{aB}	<0.0001
10	1.20±0.08 ^{aA}	1.10±0.08 ^{aA}	1.03±0.07 ^{aA}	0.77±0.12 ^{aB}	0.0167
<i>P</i> value	0.0002	0.0044	0.0100	0.0011	

Values are means ± standard deviation of three determinations; mean values with different lowercase letters in the same column and different uppercase letters in the same row are significantly different ($P < 0.05$).

Table 6. The log reductions of *S. Typhimurium* (from 10⁹ log) when incubated with phenolic extract for 6 days

Soybean varieties					
Extract concentration (%)	R07-1927	R09-349	R07-589	R08-4004	<i>P</i> value
0.5	1.20±0.14 ^{bA}	1.13±0.19 ^{bA}	0.90±0.08 ^{cAB}	0.70±0.08 ^{bB}	0.0186
1	1.73±0.05 ^{aA}	1.37±0.17 ^{bB}	0.93±0.05 ^{cC}	0.80±0.08 ^{bC}	<0.0001
2	1.73±0.05 ^{aA}	1.37±0.24 ^{bB}	1.00±0.00 ^{cC}	0.83±0.05 ^{bC}	0.0004
6	1.87±0.05 ^{aA}	1.77±0.05 ^{aAB}	1.67±0.05 ^{bB}	1.37±0.12 ^{aC}	0.0008
10	1.87±0.05 ^{aA}	1.80±0.00 ^{aA}	1.87±0.05 ^{aA}	1.47±0.12 ^{aB}	0.0013
<i>P</i> value	<0.0001	0.0067	<0.0001	<0.0001	

Values are means ± standard deviation of three determinations; mean values with different lowercase letters in the same column and different uppercase letters in the same row are significantly different ($P < 0.05$).

Table 7. The log reductions of *S. Typhimurium* (from 10^4 log) when incubated with phenolic extract for 6 days

Soybean varieties					
Extract concentration (%)	R07-1927	R09-349	R07-589	R08-4004	<i>P</i> value
0.5	1.50±0.08 ^{cA}	1.67±0.05 ^{cAB}	1.37±0.09 ^{cB}	1.40±0.08 ^{cB}	0.0197
1	1.77±0.05 ^{bA}	1.73±0.05 ^{bcA}	1.50±0.00 ^{cB}	1.40±0.08 ^{bB}	0.0003
2	1.80±0.08 ^{bA}	1.73±0.09 ^{bcA}	1.50±0.00 ^{cB}	1.67±0.05 ^{bA}	0.0105
6	1.83±0.05 ^{bA}	1.87±0.05 ^{abA}	1.80±0.14 ^{bA}	1.77±0.05 ^{abA}	0.6588
10	2.03±0.05 ^{aA}	2.00±0.08 ^{aA}	2.07±0.05 ^{aA}	1.80±0.00 ^{aB}	0.0040
<i>P</i> value	<0.0001	0.0037	<0.0001	<0.0001	

Values are means ± standard deviation of three determinations; mean values with different lowercase letters in the same column and different uppercase letters in the same row are significantly different ($P < 0.05$).

Table 8. The log reductions of *E. coli* O157:H7 (from 10^9 log) when incubated with phenolic extract for 1 day

Soybean varieties					
Extract concentration (%)	R07-1927	R09-349	R07-589	R08-4004	<i>P</i> value
0.5	0.60±0.08 ^{caA}	0.50±0.08 ^{caA}	0.47±0.05 ^{caA}	0.07±0.09 ^{bbB}	0.0006
1	0.67±0.12 ^{bcA}	0.57±0.12 ^{bcA}	0.60±0.08 ^{bcA}	0.40±0.43 ^{abA}	0.7186
2	0.67±0.05 ^{bcA}	0.63±0.09 ^{bcAB}	0.60±0.14 ^{bcAB}	0.43±0.09 ^{abB}	0.1723
6	0.77±0.05 ^{abA}	0.73±0.05 ^{abA}	0.67±0.05 ^{bA}	0.50±0.08 ^{abB}	0.0073
10	0.90±0.00 ^{aA}	0.83±0.05 ^{aA}	0.73±0.05 ^{aB}	0.53±0.05 ^{aC}	<0.0001
<i>P</i> value	0.0165	0.0185	0.0763	<0.0001	

Values are means ± standard deviation of three determinations; mean values with different lowercase letters in the same column and different uppercase letters in the same row are significantly different ($P < 0.05$).

Table 9. The log reductions of *E. coli* O157:H7 (from 10^4 log) when incubated with phenolic extract for 1 day

Soybean varieties					
Extract concentration (%)	R07-1927	R09-349	R07-589	R08-4004	<i>P</i> value
0.5	0.80±0.00 ^{dA}	0.77±0.05 ^{cA}	0.70±0.08 ^{cA}	0.67±0.09 ^{aA}	0.2503
1	0.93±0.05 ^{cA}	0.87±0.09 ^{bcAB}	0.73±0.09 ^{cAB}	0.67±0.12 ^{aB}	0.0770
2	0.93±0.05 ^{cA}	0.90±0.00 ^{bA}	0.77±0.05 ^{cB}	0.77±0.05 ^{aB}	0.0056
6	1.07±0.05 ^{bA}	1.03±0.05 ^{aA}	1.03±0.05 ^{bA}	0.77±0.12 ^{aB}	0.0970
10	1.20±0.08 ^{aA}	1.13±0.05 ^{aA}	1.20±0.08 ^{aA}	0.83±0.05 ^{aB}	0.0016
<i>P</i> value	0.0002	0.0005	<0.0001	0.3786	

Values are means ± standard deviation of three determinations; mean values with different lowercase letters in the same column and different uppercase letters in the same row are significantly different ($P < 0.05$).

Table 10. The log reductions of *E. coli* O157:H7 (from 10⁹ log) when incubated with phenolic extract for 3 days

Soybean varieties					
Extract concentration (%)	R07-1927	R09-349	R07-589	R08-4004	P value
0.5	0.70±0.00 ^{cA}	0.67±0.05 ^{cA}	0.67±0.05 ^{cA}	0.53±0.09 ^{cB}	0.0797
1	0.80±0.08 ^{cA}	0.70±0.00 ^{cB}	0.70±0.00 ^{cB}	0.57±0.05 ^{bcC}	0.0071
2	0.80±0.00 ^{cA}	0.73±0.05 ^{cAB}	0.70±0.00 ^{cBC}	0.63±0.05 ^{bcC}	0.0068
6	1.20±0.14 ^{bA}	1.10±0.08 ^{bA}	1.07±0.05 ^{bA}	0.73±0.09 ^{bB}	0.0067
10	1.97±0.05 ^{aA}	1.93±0.09 ^{aA}	1.63±0.09 ^{aB}	0.93±0.09 ^{aC}	<0.0001
P value	<0.0001	<0.0001	<0.0001	0.0032	

Values are means ± standard deviation of three determinations; mean values with different lowercase letters in the same column and different uppercase letters in the same row are significantly different ($P < 0.05$).

Table 11. The log reductions of *E. coli* O157:H7 (from 10^4 log) when incubated with phenolic extract for 3 days

Soybean varieties					
Extract concentration (%)	R07-1927	R09-349	R07-589	R08-4004	<i>P</i> value
0.5	1.03±0.05 ^{cA}	0.90±0.08 ^{bA}	0.93±0.09 ^{aA}	0.53±0.05 ^{dB}	0.0005
1	1.03±0.05 ^{cA}	0.93±0.09 ^{bA}	0.97±0.12 ^{aA}	0.63±0.12 ^{cdB}	0.0015
2	1.17±0.17 ^{cA}	1.03±0.05 ^{bA}	1.03±0.21 ^{aA}	0.70±0.00 ^{bcB}	0.0434
6	1.90±0.08 ^{bA}	1.10±0.08 ^{bB}	1.23±0.12 ^{aB}	0.80±0.00 ^{abC}	<0.0001
10	2.20±0.08 ^{aA}	2.00±0.16 ^{aA}	1.33±0.47 ^{aB}	0.90±0.08 ^{aB}	0.0033
<i>P</i> value	<0.0001	<0.0001	0.4499	0.0032	

Values are means ± standard deviation of three determinations; mean values with different lowercase letters in the same column and different uppercase letters in the same row are significantly different ($P < 0.05$).

Table 12. The log reductions of *E. coli* O157:H7 (from 10^9 log) when incubated with phenolic extract for 6 days

Soybean varieties					
Extract concentration (%)	R07-1927	R09-349	R07-589	R08-4004	<i>P</i> value
0.5	1.77±0.05 ^{cA}	1.70±0.08 ^{cA}	1.73±0.09 ^{bA}	0.87±0.05 ^{dB}	<0.0001
1	1.87±0.05 ^{cA}	1.80±0.00 ^{bcA}	1.77±0.05 ^{bA}	1.03±0.05 ^{cdB}	<0.0001
2	1.90±0.00 ^{cA}	1.87±0.05 ^{bAB}	1.77±0.12 ^{bB}	1.10±0.08 ^{cC}	0.0007
6	2.10±0.08 ^{bA}	1.93±0.05 ^{bB}	1.80±0.00 ^{bB}	1.30±0.08 ^{bC}	<0.0001
10	2.67±0.12 ^{aA}	2.23±0.09 ^{aB}	2.03±0.05 ^{aB}	1.77±0.12 ^{aC}	<0.0001
<i>P</i> value	<0.0001	<0.0001	0.0166	<0.0001	

Values are means ± standard deviation of three determinations; mean values with different lowercase letters in the same column and different uppercase letters in the same row are significantly different ($P < 0.05$).

Table 13. The log reductions of *E. coli* O157:H7 (from 10^4 log) when incubated with phenolic extract for 6 days

Soybean varieties					
Extract concentration (%)	R07-1927	R09-349	R07-589	R08-4004	<i>P</i> value
0.5	1.97±0.05 ^{dA}	2.03±0.05 ^{dA}	2.03±0.09 ^{cA}	1.53±0.12 ^{cB}	0.0009
1	2.43±0.09 ^{cA}	2.40±0.08 ^{cA}	2.03±0.05 ^{cB}	1.90±0.08 ^{cB}	0.0003
2	2.53±0.05 ^{cA}	2.43±0.05 ^{cA}	2.13±0.05 ^{cB}	1.90±0.08 ^{bcC}	<0.0001
6	3.00±0.00 ^{bA}	2.73±0.05 ^{bbB}	2.30±0.00 ^{bC}	2.10±0.00 ^{abD}	<0.0001
10	3.30±0.08 ^{aA}	3.13±0.05 ^{aB}	3.00±0.08 ^{aB}	2.17±0.05 ^{aC}	<0.0001
<i>P</i> value	<0.0001	<0.0001	<0.0001	0.0080	

Values are means ± standard deviation of three determinations; mean values with different lowercase letters in the same column and different uppercase letters in the same row are significantly different ($P < 0.05$).

Table 14. The log reductions of *C. jejuni* (from 10^9 log) when incubated with phenolic extract for 1 day

Soybean varieties					
Extract concentration (%)	R07-1927	R09-349	R07-589	R08-4004	<i>P</i> value
0.5	0.73±0.09 ^{cA}	0.63±0.09 ^{cA}	0.60±0.08 ^{bA}	0.07±0.05 ^{cB}	0.0002
1	0.80±0.00 ^{bcA}	0.67±0.05 ^{cA}	0.70±0.00 ^{bA}	0.07±0.05 ^{cB}	0.3452
2	0.83±0.00 ^{bcA}	0.80±0.05 ^{cA}	0.70±0.00 ^{bB}	0.33±0.05 ^{bcC}	<0.0001
6	0.90±0.00 ^{bA}	0.80±0.00 ^{bA}	0.87±0.05 ^{aA}	0.47±0.09 ^{bB}	<0.0001
10	1.03±0.05 ^{aA}	0.97±0.05 ^{aA}	0.97±0.05 ^{aA}	0.60±0.00 ^{aB}	<0.0001
<i>P</i> value	0.0007	0.0007	<0.0001	<0.0001	

Values are means ± standard deviation of three determinations; mean values with different lowercase letters in the same column and different uppercase letters in the same row are significantly different ($P < 0.05$).

Table 15. The log reductions of *C. jejuni* (from 10^4 log) when incubated with phenolic extract for 1 day

Soybean varieties					
Extract concentration (%)	R07-1927	R09-349	R07-589	R08-4004	<i>P</i> value
0.5	0.80±0.08 ^{dA}	0.83±0.09 ^{bA}	0.83±0.09 ^{bA}	0.27±0.09 ^{bB}	0.0006
1	0.87±0.05 ^{cdA}	0.83±0.09 ^{bA}	0.90±0.00 ^{bA}	0.33±0.12 ^{bB}	0.0003
2	0.97±0.05 ^{cA}	0.83±0.09 ^{bAB}	0.90±0.00 ^{bAB}	0.77±0.12 ^{aB}	0.1631
6	1.10±0.00 ^{bA}	1.07±0.05 ^{aA}	1.03±0.05 ^{aA}	0.87±0.12 ^{aB}	0.0440
10	1.23±0.05 ^{aA}	1.20±0.00 ^{aA}	1.13±0.05 ^{aA}	0.93±0.09 ^{aB}	0.0034
<i>P</i> value	<0.0001	0.0015	<0.0001	0.0003	

Values are means ± standard deviation of three determinations; mean values with different lowercase letters in the same column and different uppercase letters in the same row are significantly different ($P < 0.05$).

Table 16. The log reductions of *C. jejuni* (from 10⁹ log) when incubated with phenolic extract for 3 days

Soybean varieties					
Extract concentration (%)	R07-1927	R09-349	R07-589	R08-4004	P value
0.5	0.87±0.05 ^{eA}	0.93±0.09 ^{dA}	1.00±0.00 ^{cA}	0.37±0.17 ^{cB}	0.0008
1	1.03±0.05 ^{dA}	0.97±0.05 ^{cdA}	1.00±0.08 ^{cA}	0.50±0.08 ^{cB}	<0.0001
2	1.30±0.08 ^{cA}	1.10±0.00 ^{cAB}	1.03±0.12 ^{bcB}	0.77±0.09 ^{bcC}	0.0022
6	1.83±0.05 ^{bA}	1.30±0.08 ^{bbB}	1.23±0.12 ^{bB}	0.97±0.09 ^{abC}	<0.0001
10	2.07±0.05 ^{aA}	1.83±0.05 ^{abB}	1.77±0.09 ^{abB}	1.13±0.09 ^{aC}	<0.0001
P value	<0.0001	<0.0001	<0.0001	0.0002	

Values are means ± standard deviation of three determinations; mean values with different lowercase letters in the same column and different uppercase letters in the same row are significantly different ($P < 0.05$).

Table 17. The log reductions of *C. jejuni* (from 10^4 log) when incubated with phenolic extract for 3 days

Soybean varieties					
Extract concentration (%)	R07-1927	R09-349	R07-589	R08-4004	<i>P</i> value
0.5	1.00±0.08 ^{dA}	1.00±0.00 ^{cA}	0.97±0.05 ^{cAB}	0.87±0.05 ^{cB}	0.0452
1	1.23±0.05 ^{cA}	1.13±0.00 ^{cB}	1.03±0.12 ^{bcC}	0.97±0.05 ^{bC}	0.0006
2	1.37±0.05 ^{cAB}	1.50±0.16 ^{bA}	1.20±0.14 ^{bBC}	1.03±0.05 ^{bC}	0.0160
6	1.93±0.05 ^{bA}	2.03±0.05 ^{bA}	2.03±0.05 ^{aA}	1.30±0.00 ^{aB}	<0.0001
10	2.10±0.08 ^{aA}	2.07±0.09 ^{aA}	2.17±0.12 ^{aA}	1.37±0.05 ^{aB}	<0.0001
<i>P</i> value	<0.0001	<0.0001	<0.0001	<0.0001	

Values are means ± standard deviation of three determinations; mean values with different lowercase letters in the same column and different uppercase letters in the same row are significantly different ($P < 0.05$).

Chapter 5: Evaluation of soybean hull phenolic extracts on *Salmonella* Typhimurium, *E coli*, and *Campylobacter jejuni* attached to chicken skin.

5.1 Introduction

Chicken is a very popular food commodity, which has a high range of consumption in almost all countries. People prefer to eat chicken for its good taste, low cost, low fat content, and high nutritional value (Mataragas *et al.*, 2008). However, food from animal sources such as chicken have the highest percentage of cause for foodborne diseases (Todd, 1996; Petersen & James, 1998). The raw chicken skin can be contaminated by pathogens during production, processing, distribution, retail marketing, handling and preparation (Zhao *et al.*, 2001). A wide variety of microorganisms are present on the skin of chilled poultry as it provides optimal conditions for growth and survival. But the presence of any kind of bacteria in or on the surface of any kind of meat is not desirable with the exception of fermented meat products (ICMSF – International Commission on Microbiological Specification for Foods, 2005).

Consumption of poultry meat has been on the rise in recent years, which might have led poultry to be implicated in about 17-18 % of foodborne illnesses (Painter *et al.*, 2013). Raw poultry meat is often contaminated with bacteria such as *Salmonella*, *E coli*, *Campylobacter*, *Arcobacter*, *Listeria*, *Staphylococcus* and *Clostridium* (Doležalová *et al.*, 2010). Some of these bacteria such as *Salmonella* and *Campylobacter* are able to survive in the feather follicles or on the skin of poultry even after the different treatments during processing (Todd, 1996; Zhang *et al.*, 2013; Chaine *et al.*, 2013). Research around the world indicate that *Campylobacter*, *Salmonella*, and *E coli* are present in fresh meat and poultry (Todd, 1996). Scallan *et al* (2011) reported that each year about 850,000 people are infected by *Campylobacter jejuni*, 1.03 million

infections by different species of *Salmonella* including *S. Typhimurium*, and 206,000 by different kinds of pathogenic *Escherichia coli*, including *E. coli* O157:H7.

Despite methods to reduce or eliminate bacterial foodborne pathogens, foodborne illnesses still continue to pose a major threat to public health (CDC, 2016). Considering the fact that chicken belongs to the group of perishable foods, this problem pushes the researcher to find solutions to increase the shelf-life extension of chicken products (Leistner, 1995; Chouliara *et al.*, 2006). Natural antimicrobials have started slowly replacing addition of traditional antimicrobials such as different antibiotics to food and food products due to increasing consumer awareness (Cetin-Karaca & Newman, 2015). So there is an increasing demand, to determine the minimum inhibitory concentrations (MIC) of plant derived phenolics (Phanthong *et al.*, 2013). Phenolic extracts, which are natural antimicrobials, have the ability to inhibit the human pathogen (Cetin-Karaca, 2011). Oliveira *et al.* (2008) indicated that phenolic extracts from the walnut (*Juglans regia* L.) green husks have high antimicrobial activity against several types of bacteria. Additionally, researchers showed that phenolic fractions of gelam and coconut honeys have potent antimicrobial activities (Aljadi & Yusoff., 2003). Studies have shown that the extracts of blueberry as well as muscadine were able to inhibit pathogens like *Listeria monocytogenes* and *Salmonella* Enteritidis (Park *et al.*, 2011).

The objective of this study was to evaluate the inhibitory effects of soybean hull phenolic extracts on *Salmonella* Typhimurium, *E. coli*, and *Campylobacter jejuni* attached to chicken skin.

5.2 Materials and methods:

Materials

Agar slant cultures of *E. coli* O157:H7 (GFP-labeled ED 14) *Salmonella* Typhimurium (ATCC 14028) was provided by Dr. Johnson, Center for Food Safety Research Laboratory,

University of Arkansas. *Campylobacter jejuni* NCTC 11168 was provided by Dr. Kwon, Department of Poultry Science, University of Arkansas. XLT4 *Salmonella* selective agar, MacConkey-Sorbitol agar, and Mueller Hinton agar media were purchased from Difco™ (a division of Becton, Dickinson and Co., USA). Nalidixic acid was purchased from VWR International, Inc (Suwanee, GA, USA). Fresh chickens were obtained from Tyson Foods Inc. The phenolic extract from the hull of the black soybean (R07-1927) was used because it produced the maximum inhibitory.

Preparation of culture:

Ten µl of frozen stock cultures of *Salmonella* Typhimurium, *Escherichia coli* O157:H7 and *Campylobacter jejuni* were used to inoculate in 10 mL of brain heart infusion homogenate (BHI) broth for *Salmonella* Typhimurium, *Escherichia coli*, and Mueller Hinton broth (MH) for *Campylobacter jejuni* at 200 rpm agitating incubator (Edison NJ, U.S.A.) Cultures of *Salmonella* and *E coli* were incubated at 37 °C for 24 h, while *C. jejuni* cultures were incubated for 48 h in a micro-aerobic atmosphere consisting of 85% nitrogen, 10% carbon dioxide, and 5% oxygen. After completion of incubation, the cultures (10 µl) from the first passage were passed into 10 mL of fresh respective broth. The cultures were incubated under conditions similar to the first passage to make sure that the culture of the bacterial was in the exponential growth phase. Cultures from second passage were used to inoculate agar plates with 100 ng/ml nalidixic acid for *S. Typhimurium* and *E. coli* while for *C. jejuni* agar plates without nalidixic acid was used. From the agar plates colonies were again passed twice in broth cultures as described above, to obtain a high and stable concentration of bacteria.

Extraction of Phenolics:

The extraction of total phenolic compounds using ethanol is described in chapter 3.

Investigation of antimicrobial activity of the phenolic extracts on chicken skin

The total phenolic was found to be the highest for the black colored variety (4.29 mg of CAE/g DW) out of the 4 different colored soybean hulls tested. Hence only this extract was used at two different concentrations for the chicken skin studies: a lowest concentration of 0.5 % and a higher concentration of 2%, which did not produce any color, changes on the meat. Skin from a fresh chicken was cut into 54 pieces of approximately 2 g size with a sterile knife. Altogether skin pieces from three chickens were used for the experiment as the effect of extract for 3 time periods of storage (1, 3 and 6 days) was tested. Individual chicken skin pieces in duplicate were inoculated with the bacterial cultures (100µl/ piece) having two concentrations (10^9 and 10^4 cfu/mL) and were kept in the refrigerator at 4°C for 15 min to promote bacterial attachment. The samples were then dipped in 100 µl of the black soybean hull extract (0.5 % and 2%) for 15 min at 4°C and then placed in stomacher bags and stored at 4°C for 1, 3 and 6 days. After storage for each time period, the stomacher bags with individual chicken skin pieces were taken out, 10 ml of PBS added and stomached for 10 min at 8 strokes/sec. Stomached samples were serially diluted using PBS and plated on to differential agar plates for the different bacteria which were as follows: (1) XLT-4 (Xylos Lysine Tergitol 4) agar plates with 25ng/ ml nalidixic acid for *S. Typhimurium* (2) SMAC (Sorbitol MacConkey) agar plates with 25ng/ ml nalidixic acid for *E. coli* and (3) Mueller Hinton (MH) agar plates for *C. jejuni*. All the agar plates for enumeration of *S. Typhimurium* and *E. coli* were incubated at 37°C for 24h (Edison, N.J., U.S.A.), while the plates for *C. jejuni* were incubated at 42°C for 48h microaerobically and colonies were counted to determine the bacterial log reductions.

Statistical analysis

Three repetitions of the experiment were done with triplicate samples. The data was analyzed by one-way analysis of variance to determine the analysis of variance ((ANOVA)) using the JMP (John's Macintosh Product) 7.0 software (SAS Inst. Inc., Cary, NC, U.S.A.). The significant difference between results were estimated at $p < 0.0001$

5.3 Results and Discussion

Many studies reported that natural alternatives such as plant derived antimicrobials have the ability to reduce bacterial loads on chicken skin (Lowenthal *et al.*, 1999; Guo *et al.*, 2004; Doležalová *et al.*, 2009; Yang & Choct, 2009; Piskernik *et al.*, 2011). In this study, phenolic extracts from the hulls of black soybean (R07-1927) was investigated as a natural plant derived antimicrobial against *Salmonella* Typhimurium, *E coli*, and *Campylobacter jejuni* attached to chicken skin. From the experiments for determining the inhibitory effects of soybean hull phenolic extracts in bacterial cultures, we found that the black soybean (R07-1927) hull extract produced the maximum inhibitory effects. We used 5 different concentrations (0.5, 1, 2, 6 and 10%) for determining inhibitory effects of the extracts towards bacteria in cultures and found that the maximum reductions were produced by the 10% concentration of the extract. However with preliminary studies we found that the two higher concentrations of 6 % and 10 % produced color changes on chicken skin. So for determining the inhibitory effect of extracts on bacteria attached to chicken skin we used the low concentration of 0.5% and 2% which did not produce any color changes on the meat. Also since we could not detect any growth of *C. jejuni* after 6 days even in cultures without treatment, this bacteria was not included for the chicken skin study.

The effects of the two different concentrations of R07-1927 hull extract for 1, 3, and 6 days of storage on *S. Typhimurium* and *E coli* attached to chicken skin, are shown in Tables 18 -

19. The extract at 2% concentration was able to produce around 1.18 and 1.39 log reductions for *Salmonella* on days 3 and 6 respectively, whereas for *E coli* the log reductions were 1.09 and 1.24. We also found that the 2% phenolic extract was more effective than the 0.5 % concentration for *Salmonella* Typhimurium ($P = 0.0102, 0.0072$ and 0.0158) after incubation for all the 3 time periods of storage (1, 3 and 6 days). However no significant differences between the two concentrations was seen on *E coli* log counts ($P = 0.05, 0.2291$ and 0.1402) at any time points tested. Similar to the effects produced by the extracts on bacteria in cultures, here also we found that the two bacteria reacted differently to the same extract, which might be due to the differences in their cell structures. Previous studies also have observed differences in inhibitory actions even between strains of bacteria within the same family when treated with plant derived phenolic compounds (Cetin-Karaca & Newman, 2015). Also the total phenolic extracts might be containing some compounds, which may be interfering with the action of the phenolic compounds. Hence separation of individual constituents from the total phenolic extracts and their antimicrobial effects warrants further studies.

5.4 Conclusion

The present study showed differences in the inhibitory effects of the phenolic extract from the black soybean hull on bacteria attached to chicken skin. The highest log reduction was observed after incubation for 6 days with 2% concentration of the phenolic extract, with log reductions of 1.39 for *Salmonella* and 1.24 for *E coli*. Many types of hurdle techniques are being employed in poultry processing to reduce/ eliminate foodborne bacteria. Yet, chicken skin still harbors different pathogenic species of bacteria known to produce foodborne diseases in humans. Due to increasing awareness among consumers, natural antimicrobials have started replacing

traditional antimicrobials in food and food products. In this present scenario, natural antimicrobials such as soybean hull extracts could be made use of to reduce bacteria in foods.

Table 18. Effect of R07-1927 (black) soybean hull phenolic extract on *S. Typhimurium* attached to chicken skin

Phenolic extract concentration (%)	Log Reduction			
	Day 1	Day3	Day 6	<i>P</i> value
0.5	0.32±0.02 ^{bC}	1.00±0.03 ^{bB}	1.18±0.05 ^{bA}	<0.0001
2	0.43±0.06 ^{aB}	1.18±0.05 ^{aA}	1.39±0.03 ^{aA}	<0.0001
<i>P</i> value	0.0102	0.0072	0.0158	

*Strains of *Salmonella*

Values are means ± standard deviation of three determinations; mean values with different lowercase letters in the same column and different uppercase letters in the same row are significantly different ($P < 0.05$).

Table 19. Effect of R07-1927 (black) soybean hull phenolic extract on *E. coli* attached to chicken skin

Phenolic extract concentration (%)	Log Reduction			
	Day 1	Day3	Day 6	<i>P</i> value
0.5	0.39±0.12 ^{bB}	0.99±0.03 ^{aA}	1.13±0.02 ^{aA}	<0.0001
2	0.52±0.02 ^{aC}	1.09±0.02 ^{aB}	1.24±0.06 ^{aA}	<0.0001
<i>P</i> value	0.0500	0.2291	0.1402	

*Strains of *E coli*

Values are means ± standard deviation of three determinations; mean values with different lowercase letters in the same column and different uppercase letters in the same row are significantly different ($P < 0.05$).

Chapter 6: A preliminary study on the Investigation of the ACE-I inhibitory activity of the phenolic extracts from hulls of four different colored soybean varieties (R07-1927, R07-589, R09-349, and R08- 4004)

6.1 Introduction

Soybean seed hulls contain several compounds such as protein, fiber, iron, lignin, trypsin and phenolics, which are found to have a variety of health benefits (Alvarez *et al.*, 1997; Sessa and Wolfe, 2001; Murray-Kolb *et al.*, 2003; Xu and Chang 2008). Phenolics have the ability to serve as Anti Angiotensin-I Converting Enzyme (ACE-I) inhibitory activities. (Ranilla *et al.*, 2010) ACE-I inhibitions play a significant role in controlling high blood pressure by the renin-angiotensin system (Mullally *et al.*, 1996). According to the American Heart Association and the Center for Disease Control and Prevention, 610,000 people die of heart-related diseases in the U.S each year (Mozaffarian *et al.*, 2016). Hypertension is a major risk factor for causing heart disease. It is known that antihypertensive activity is related to the inhibition of Angiotensin-I converting-enzyme (ACE-I) catalytic activity (Lee *et al.*, 2010). ACE-I is known to be responsible for vascular constriction, thereby causing hypertension (Liesmaa, 2010). Renin-angiotensin system (RAS) from the liver transforms the angiotensinogen to angiotensin I (histidyl-leucine dipeptide), which in turn is transported to the lungs. ACE-I changes the inactive form into angiotensin-II (potent vasoconstrictor octapeptide) and activates the vasodilator bradykinin (Skeggs *et al.*, 1956; Mallikarjun Gouda *et al.*, 2006; Hong *et al.*, 2008). Additionally, angiotensin II catalyzes the synthesis and release of aldosterone, which causes hypertension by promoting sodium retention in the distal tubules (Lieberman, 1975). Inhibition of the ACE activity is the best method that used in treatment of increasing the blood pressure (Erdös& Skidgel., 1987).

Drugs such as captopril and enalapril, are administered as antihypertensive agents, which inhibit the angiotensin I-converting enzyme (ACE) and leading to a decrease in blood pressure and reduce the risk of hypertension complications. However, those synthetic treatments have side effects, including dry cough, skin rashes, and allergic reactions (Choi *et al.*, 2001; Hong *et al.*, 2008; García *et al.*, 2013). Of these side affects, reaserches have been prompted to find ACE inhibitors from natural sources. Many studies indicate that there are numbers of compounds in plants such as phenolic compounds, peptides, oligosaccharides and amino acid analogues have been shown the inhibitor of ACE activity (Liu *et al.*, 2004; Actis-Goretta *et al.*, 2006; Je *et al.*, 2006).

Researchers have shown many different ACE-I inhibiting compounds from soybean sources (Chiang *et al.*, 2005; Yang *et al.*, 2011; Lassissi *et al.*, 2014). However, no literature infomation is available in investigating the effect of various soyben colored hull phnolic extracts in the inhibition of ACE -1. Hence, the objective of this study was to investigate the ACE-I inhibitory activity of the extracts.

6.2 Materials and methods

Materials

Monosodium phosphate monohydrate, disodium phosphate heptahydrate, sodium chloride, hydrochloric acid and acetic acid were purchased from VWR International, Inc. (Suwanee, GA, USA). ACE stock solution, hippuryl-L-histidyl-L-leucine (HHL) and captopril were purchased from Sigma-Aldrich, Inc.

Determination of Angiotensin-converting-enzyme inhibitor (ACE inhibitory) activity:

Two types of solvents were prepared for the experiment, Solvent A and Solvent B. Solvent A was used to prepare the extract sample, ACE and Solvent B. Solvent B was used to

prepare hippuryl-L-histidyl-L-leucine (HHL). Solvent A (1L of 0.1 M phosphate buffer (PB) of pH 8.3) was prepared by adding 0.489 g of monosodium phosphate monohydrate (MPB) and 25.851 g disodium phosphate, heptahydrate in 1 L DI water and pH was adjusted to 8.3 using sodium hydroxide. Solvent B (100ml of 0.5M NaCl in 0.1 M PB of pH 8.3) was prepared by adding 2.925 g NaCl to 100 mL of Solvent A. One ml of 125 mU / mL of ACE was prepared by mixing 125 uL of ACE stock solution (1U/MI) and 875 uL of Solvent A. Five ml of 12.5 mM hippuryl-L-histidyl-L-leucine (HHL) was prepared by mixing 0.0268g of HHL to 5 mL of Solvent B.

One 1 mL of the extract solution was prepared by adding 0.01 g of extract/ Captopril to 1mL of Solvent A. The samples were prepared by taking 50 uL of the extract solution in a screw cap vial to which 50 uL of ACE solution and 150 uL of HHL solution was added, vortexed and incubated at 37°C in a water bath for 1 h. The samples were done in triplicates. 250 uL of 1N HCl was added to stop the reaction, then 1.0 mL of ethyl acetate was added to extract the hippuric acid liberated from HHL by ACE activity. The tubes were centrifuged (centrifuge model J2-21, Beckman, Fullerton, Calif., U.S.A.) at 15,000 rpm for 5 min. The supernatant of ethyl acetate extract (0.75 mL) was evaporated to dryness in a water bath at 90 °C, and the liberated hippuric acid from the extract was dissolved in 1.0 mL of DI water. A blank was prepared similarly using PB pH 8.3 except that the HCl was added to the mixture before the addition of the enzyme. The amount of hippuric acid liberated was measured spectrophotometrically at 228 nm. Inhibition was calculated as follows: inhibition (%) = $[1 - (\text{absorbance in the presence of hydrolysate}) / (\text{absorbance in the absence of hydrolysate})] \times 100$

Statistical analysis

The experiments were conducted with triplicates samples. The data was analyzed by one-way analysis of variance to determine the analysis of variance ((ANOVA)) using the JMP (John's Macintosh Product) 7.0 software (SAS Inst. Inc., Cary, NC, U.S.A.). The significant difference between results were estimated at $p < 0.0001$.

6.3 Results and Discussion

For the ACE inhibitory activity, hippuryl-L-histidyl-L-leucine (HHL) is used as the substrate for ACE, as it stimulates the conversion of HHL to hippuric acid and the dipeptide, histidyl-leucine. Thus, there is a relationship between ACE inhibitory activity and the extent of hippuric acid release. If the amounts of hippuric acid decrease, the inhibitory activities of ACE increase (Abdullah *et al.*, 2011). Angiotensin I converting enzyme (ACE) produces angiotensin II which is a powerful vasoconstrictor and leading to hypertension (Hansen *et al.*, 1996; Oboh *et al.*, 2014). The research on ACE inhibitors to inhibit the angiotensin II became widely popular since the discovery of ACE inhibitors in snake venom (Villar *et al.*, 1986). The ACE inhibitory activity of phenolic extracts from plants have been reported in previous studies (Lee *et al.*, 2004; Hagiwara *et al.*, 2005; Abdullah *et al.*, 2011; Oboh *et al.*, 2013).

The results of the inhibition of angiotensin I converting enzyme (ACE) activity of phenolic extracts from four different varieties of soybean seed hulls are presented in Figure 5. The ACE-I inhibitory activities ranged from 21% to 52%. The highest ACE-I inhibitory activity among these varieties was for the extract from R07-1927 (52%) while lower ACE-I inhibitory activities was for the R08- 4004 extract (21%). No significant difference ($P = 0.0011$) between R07-589 (37%) and R09-349 (34%) was found in our study. The significant increase in ACE-I inhibitory activity of R07-1927 (black) might be due to its highest amount of phenolics.

Phommalath *et al.*, 2014 reported that black soybeans hulls have traditional medicinal value as it contains natural phenolic compounds such as proanthocyanidins and anthocyanins. The effect of the phenolic extracts from the soybean hulls on ACE activity was directly proportional to the amount of total phenolics in each type of hull extract. Research has shown that phenolics can modify the structure of ACE enzyme and reduce its activity by interacting with the disulphide bridges present on the surface of the enzyme (John & Schmieder, 2003). Therefore, inhibition of ACE by phenolics of the soybean hulls can be considered as a useful treatment in inhibition of hypertension. The results of our study with phenolic extracts of selected soybean hulls are in agreement with earlier reports on inhibition of ACE by phenolic extracts of bitter leaf (Saliu *et al.*, 2012) soybean (Ademiluyi & Oboh, 2013) and *Allium sativum* from garlic (Oboh *et al.*, 2013).

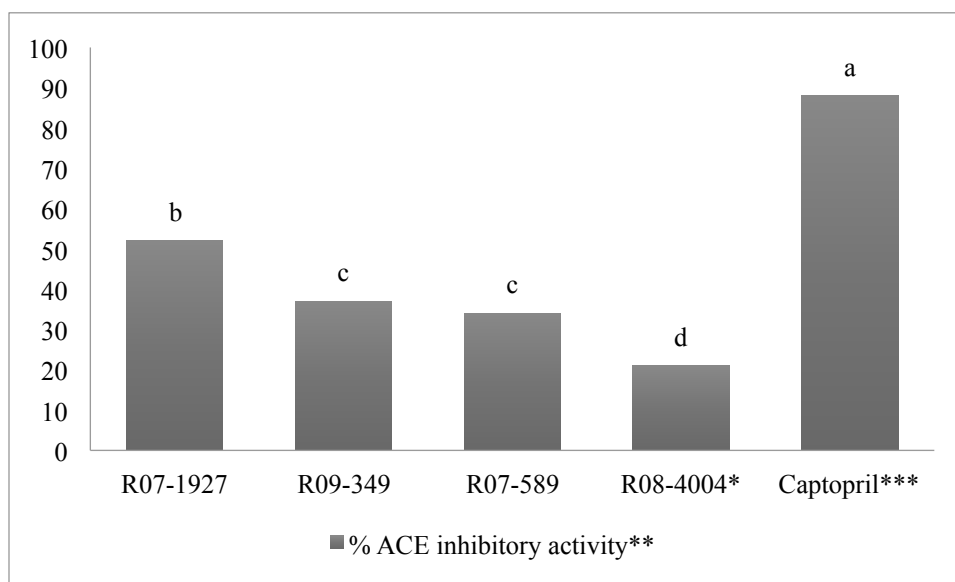
The phenolic extracts of the four different soybean hulls exhibited ACE-I inhibitory activity at a significantly lower level in comparison to the activity of Captopril ($P < 0.0001$). The effectiveness at varying amounts of the extracts needs further investigation. The phenolic extracts from soybean hulls has the potential to be used as an alternative choice in the treatment and inhibition of hypertension with lower cost and without / minimal side effects.

6.4 Conclusion

This preliminary study demonstrated that phenolic extracts from soybean hulls can inhibit angiotensin 1 converting enzyme (ACE). However the study was done with the total phenolics extracted from the soybean hulls, which might be containing some compounds which may be interfering with the action of the phenolic compounds in inhibiting ACE-I. So further studies with individual phenolic compounds separated from the total phenolics may give enhanced inhibitory activity of ACE-I. In this study, the highest ACE-I inhibitory activities were shown by

R07-1927 (black) extract while the lowest was for the R08- 4004 (yellow) extract. The significant increase in ACE-I inhibitory activity of R07-1927 (black) might be due to its higher content of total phenolics.

Figure 5. ACE-I inhibitory activities of phenolic extract from four varieties of soybean hulls



* Conventional soybean variety

**Values are means \pm standard deviation of three replicate determinations; Values with the same letter are not significantly different ($P > 0.05$).

***Captopril (0.01g/ml) was used as positive control inhibitor sample.

References

- Abascal, K., Ganora, L., & Yarnell, E. (2005). The effect of freeze-drying and its implications for botanical medicine: a review. *Phytotherapy Research*, 19(8), 655-660.
- Abdullah, N., Ismail, S. M., Aminudin, N., Shuib, A. S., & Lau, B. F. (2011). Evaluation of selected culinary-medicinal mushrooms for antioxidant and ACE inhibitory activities. *Evidence-Based Complementary and Alternative Medicine*, 2012.
- Actis-Goretta, L., Ottaviani, J. I., & Fraga, C. G. (2006). Inhibition of angiotensin converting enzyme activity by flavanol-rich foods. *Journal of agricultural and food chemistry*, 54(1), 229-234.
- Ademiluyi, A. O., & Oboh, G. (2013). Soybean phenolic-rich extracts inhibit key-enzymes linked to type 2 diabetes (α -amylase and α -glucosidase) and hypertension (angiotensin I converting enzyme) in vitro. *Experimental and Toxicologic Pathology*, 65(3), 305-309.
- Aljadi, A. M., & Yusoff, K. M. (2003). Isolation and identification of phenolic acids in Malaysian honey with antibacterial properties. *Turkish Journal of Medical Sciences*, 33(4), 229-236.
- American Heart Association. (2014). Understand your risk for high blood pressure. Retrieved July, 1, 2015.
- American Heart Association. (2015). Heart Disease and Stroke Statistics—At-a-Glance.
- Anonymous. (2003). Annual report on zoonoses in Denmark 2002. *The Danish Zoonosis Centre, Copenhagen, Denmark*.
- Astadi, I. R., Astuti, M., Santoso, U., & Nugraheni, P. S. (2009). In vitro antioxidant activity of anthocyanins of black soybean seed coat in human low density lipoprotein (LDL). *Food chemistry*, 112(3), 659-663.
- Atterbury, R. J., Connerton, P. L., Dodd, C. E., Rees, C. E., & Connerton, I. F. (2003). Application of host-specific bacteriophages to the surface of chicken skin leads to a reduction in recovery of *Campylobacter jejuni*. *Applied and environmental microbiology*, 69(10), 6302-6306.
- Alvarez, P. J. C., Krzyzanowski, F. C., Mandarino, J. M. G., & França Neto, J. B. (1997). Relationship between soybean seed coat lignin content and resistance to mechanical damage. *Seed Science and Technology*, 25(2), 209-214.
- Ayala-Zavala, J. F., Rosas-Domínguez, C., Vega-Vega, V., & González-Aguilar, G. A. (2010). Antioxidant Enrichment and Antimicrobial Protection of Fresh-Cut Fruits Using Their Own Byproducts: Looking for Integral Exploitation. *Journal of food science*, 75(8), R175-R181.

- Azizi, M., & Ménard, J. (2004). Combined blockade of the renin-angiotensin system with angiotensin-converting enzyme inhibitors and angiotensin II type 1 receptor antagonists. *Circulation*, 109(21), 2492-2499.
- Berrang, M. E., Buhr, R. J., & Cason, J. A. (2000). Campylobacter recovery from external and internal organs of commercial broiler carcass prior to scalding. *Poultry Science*, 79(2), 286-290.
- Berger, C.N., Sodha, S.V., Shaw, R.K., Griffin, P.M., Pink, D., Hand, P., Frankel, G. (2010). Fresh fruits and vegetables as vehicles for the transmission of human pathogens. *Environmental Microbiology*, 12: 2385-2397.
- Bolanho, B. C., & Beléia, A. D. P. (2011). Bioactive compounds and antioxidant potential of soy products. *Alimentos e nutriçao*, 22(4), 539-546.
- Bruhn, C. M., & Schutz, H. G. (1999). Consumer food safety knowledge and practices. *Journal of Food Safety*, 19(1), 73-87.
- Bryan, F. L., & Doyle, M. P. (1995). Health risks and consequences of *Salmonella* and *Campylobacter jejuni* in raw poultry. *Journal of Food Protection*®, 58(3), 326-344.
- Buswell, C. M., Herlihy, Y. M., Lawrence, L. M., McGuiggan, J. T., Marsh, P. D., Keevil, C. W., & Leach, S. A. (1998). Extended survival and persistence of *Campylobacter*spp. in water and aquatic biofilms and their detection by immunofluorescent-antibody and-rRNA staining. *Applied and Environmental Microbiology*, 64(2), 733-741.
- Buzby, J. C., Frenzen, P. D., & Rasco, B. (2001). Product liability and microbial foodborne illness. US Department of Agriculture, Economic Research Service.
- Carbonaro, M., Mattera, M., Nicoli, S., Bergamo, P., & Cappelloni, M. (2002). Modulation of antioxidant compounds in organic vs conventional fruit (peach, *Prunus persica* L., and pear, *Pyrus communis* L.). *Journal of agricultural and food chemistry*, 50(19), 5458-5462.
- Cavanagh, H. M., Hipwell, M., & Wilkinson, J. M. (2003). Antibacterial activity of berry fruits used for culinary purposes. *Journal of medicinal food*, 6(1), 57-61.
- Cavitte, J. (2003). Present and future control of food-borne pathogens in poultry; revision of the European Community legislation on zoonoses. In Proceedings of the XVI European Symposium on the Quality of Poultry Meat (p. 4658).
- CDC (2009) Recommendations for Diagnosis of Shiga Toxin--Producing *Escherichia coli* Infections by Clinical Laboratories Accessed from: <https://www.cdc.gov/mmwr/preview/mmwrhtml/rr5812a1.htm>

- CDCa. (2014) Campylobacter General Information. Accessed from:
<http://www.cdc.gov/nczved/divisions/dfbmd/diseases/campylobacter/>
- CDCb. (2014). CDC estimates of foodborne illness in the United States. Accessed from:
<http://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html> on January 8, 2014.
- CDCa. (2015). NCHS. Underlying Cause of Death 1999-2013 on CDC WONDER Online Database, released 2015. Data are from the Multiple Cause of Death Files, 1999-2013, as compiled from data provided by the 57 vital statistics jurisdictions through the Vital Statistics Cooperative Program. Accessed Feb. 3, 2015. Accessed from:
<https://www.cdc.gov/heartdisease/facts.htm>
- CDCb. (2015). CDC estimates of foodborne illness in the United States. Accessed from:
<http://www.ers.usda.gov/media/1837791/eib140.pdf> on May, 2015.
- CDCa. (2016). Eight Multistate Outbreaks of Human *Salmonella* Infections Linked to Live Poultry in Backyard Flocks. Accessed from: <http://www.cdc.gov/salmonella/live-poultry-05-16/index.html>
- CDCb. (2016) Reports of *Salmonella* Outbreak Investigations Accessed from:
<https://www.cdc.gov/salmonella/>
- Cetin-Karaca, H. (2011). Evaluation of natural antimicrobial phenolic compounds against foodborne pathogens.
- Cetin-Karaca, H., & Newman, M. C. (2015). Antimicrobial Efficacy of Natural Phenolic Compounds against Gram Positive Foodborne Pathogens. *Journal of Food Research*, 4(6), 14.
- Chaine, A., Arnaud, E., Kondjoyan, A., Collignan, A., & Sarter, S. (2013). Effect of steam and lactic acid treatments on the survival of *Salmonella* Enteritidis and *Campylobacter jejuni* inoculated on chicken skin. *International journal of food microbiology*, 162(3), 276-282.
- Chappell, L., Kaiser, P., Barrow, P., Jones, M. A., Johnston, C., & Wigley, P. (2009). The immunobiology of avian systemic salmonellosis. *Veterinary immunology and immunopathology*, 128(1), 53-59.
- Chiang, W.D., Tsou, M.J., Tsai, Z.Y., Tsai, T.C. (2005). Angiotensin I-converting enzyme inhibitor derived from soy protein hydrolysate and produced by using membrane reactor. *Food Chemistry*, 98: 725-732.
- Chidambara Murthy, K. N., Singh, R. P., & Jayaprakasha, G. K. (2002). Antioxidant activities of grape (*Vitis vinifera*) pomace extracts. *Journal of agricultural and food chemistry*, 50(21), 5909-5914.
- Chobanian, A. V., Bakris, G. L., Black, H. R., Cushman, W. C., Green, L. A., Izzo, J. L., ... & Rocella, E. J. (2003). Seventh report of the joint national committee on prevention,

- detection, evaluation, and treatment of high blood pressure. *Hypertension*, 42(6), 1206-1252.
- Choi, H. S., Cho, H. Y., Yang, H. C., Ra, K. S., & Suh, H. J. (2001). Angiotensin I-converting enzyme inhibitor from *Grifola frondosa*. *Food Research International*, 34(2), 177-182.
- Conner, D. E., Davis, M. A., & Zhang, L. (2001). Poultry-borne pathogens: plant considerations. *CRC Press, Boca Raton, FL*, 137-158.
- Cormican, M., DeLappe, N., O'Hare, C., Doran, G., Morris, D., Corbett-Feeney, G., ... & Moore, J. (2002). *Salmonella* enterica serotype Bredeney: antimicrobial susceptibility and molecular diversity of isolates from Ireland and Northern Ireland. *Applied and environmental microbiology*, 68(1), 181-186.
- Chouliara, E., Kontominas, M. G., Singh, V. K., Govil, J. N., Ahmad, K., & Sharma, R. K. (2007). Combined effect of thyme essential oil and modified atmosphere packaging to extend shelf-life of fresh chicken meat. *Natural products*, 1, 423-441.
- Crim, S. M., Iwamoto, M., Huang, J. Y., Griffin, P. M., Gilliss, D., Cronquist, A. B., ... & Lathrop, S. (2014). Incidence and trends of infection with pathogens transmitted commonly through food—Foodborne Diseases Active Surveillance Network, 10 US sites, 2006–2013. *MMWR Morb Mortal Wkly Rep*, 63(15), 328-32.
- Guo, F. C., Williams, B. A., Kwakkel, R. P., Li, H. S., Li, X. P., Luo, J. Y., ... & Verstegen, M. W. A. (2004). Effects of mushroom and herb polysaccharides, as alternatives for an antibiotic, on the cecal microbial ecosystem in broiler chickens. *Poultry Science*, 83(2), 175-182.
- Cuppett, S., Schnepf, M., & Hall, C. (1997). Natural antioxidants-are they a reality? In: F. Shahidi, *Natural antioxidants: chemistry, health effects, and applications* (pp. 12-24). Illinois: AOCS Press, Illinois.
- Cushnie, T. T., & Lamb, A. J. (2005). Antimicrobial activity of flavonoids. *International journal of antimicrobial agents*, 26(5), 343-356.
- Dai, J., & Mumper, R. J. (2010). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules*, 15(10), 7313-7352.
- D'Archivio, M., Filesi, C., Di Benedetto, R., Gargiulo, R., Giovannini, C., & Masella, R. (2007). Polyphenols, dietary sources and bioavailability. *Annali-Istituto Superiore di Sanita*, 43(4), 348.
- Deep, A., Chaudhary, U., & Gupta, V. (2011). Quorum sensing and bacterial pathogenicity: from molecules to disease. *Journal of laboratory physicians*, 3(1), 4.
- DeWaal, C. S. (1996). *Playing Chicken: The Human Cost of Inadequate Government Regulation*

of the Poultry Industry. *Center for Science in the Public Interest*.

- Dingle, K. E., Colles, F. M., Wareing, D. R. A., Ure, R., Fox, A. J., Bolton, F. E., ... & Maiden, M. C. J. (2001). Multilocus sequence typing system for *Campylobacter jejuni*. *Journal of clinical microbiology*, 39(1), 14-23.
- Drugs for hypertension (2012). Treatment Guidelines From The Medical Letter, 10(113): 1-10
- Doležalová, M., Molatová, Z., Buňka, F., Březina, P., & Marounek, M. (2010). Effect of organic acids on growth of chilled chicken skin microflora. *Journal of food safety*, 30(2), 353-365.
- Duenas, M., Hernandez, T., & Estrella, I. (2006). Assessment of in vitro antioxidant capacity of the seed coat and the cotyledon of legumes in relation to their phenolic contents. *Food Chemistry*, 98(1), 95-103.
- Erdős, E. G., & Skidgel, R. A. (1987). The angiotensin I-converting enzyme. Laboratory investigation; *a journal of technical methods and pathology*, 56(4), 345.
- European Technology Platform on Nanomedicine. (2005). Vision paper and basis for a strategic research agenda for nanomedicine. European Commission Luxembourg, Office for Official Publications of the European Commission, ISBN 92-894-9599-5
- Farley, T.A., Dalal, M.A., Mostashari, F., & Frieden, T.R. (2010). Deaths preventable in the US by improvements in the use of clinical preventive services. *American Journal Preventive Medicine*, 38(6): 600-609.
- Farquharson, C. A., & Struthers, A. D. (2000). Spironolactone increases nitric oxide bioactivity, improves endothelial vasodilator dysfunction, and suppresses vascular angiotensin I/angiotensin II conversion in patients with chronic heart failure. *Circulation*, 101(6), 594-597.
- Fattouch, S., Caboni, P., Coroneo, V., Tuberoso, C. I., Angioni, A., Dessi, S., ... & Cabras, P. (2007). Antimicrobial activity of Tunisian quince (*Cydonia oblonga* Miller) pulp and peel polyphenolic extracts. *Journal of Agricultural and Food Chemistry*, 55(3), 963-969.
- Feng, P. (2012). Pathogenic *Escherichia coli* group. In: Keith Lampel, Sufian Al-Khaldi, Susan Cahill, editors. *Bad Bug Book- Foodborne Pathogenic Microorganisms and Natural Toxins*. 2nd ed. Center for Food Safety and Applied Nutrition (CFSAN) of the Food and Drug Administration (FDA), U.S. Department of Health and Human Services. p 67-81.
- Floridi, S., Montanari, L., Marconi, O., & Fantozzi, P. (2003). Determination of free phenolic acids in wort and beer by coulometric array detection. *Journal of agricultural and food chemistry*, 51(6), 1548-1554.

- Foodsafety (2013). *E coli*: Accessed on July 20, 2016
<http://www.foodsafety.gov/poisoning/causes/bacteriaviruses/ecoli/>
- Frenzen, P. D., Drake, A., Angulo, F. J., & Emerging Infections Program FoodNet Working Group. (2005). Economic cost of illness due to Escherichia coli O157 infections in the United States. *Journal of Food Protection*®, 68(12), 2623-2630.
- Fryar, C. D., Chen, T., & Li, X. (2012). Prevalence of uncontrolled risk factors for cardiovascular disease: United States, 1999–2010. *NCHS data brief*, 103, 1-8.
- García, M.C., Puchalska, P., Esteve, C., & Marina, M.L. (2013). Vegetable foods: A cheap source of proteins and peptides with antihypertensive, antioxidant, and other less occurrences bioactivities. *Talanta*, 106: 328-349.
- Gnanasambandam, R., & Proctor, A. (1999). Preparation of soy hull pectin. *Food Chemistry*, 65(4), 461-467.
- Goode, D., Allen, V. M., & Barrow, P. A. (2003). Reduction of experimental *Salmonella* and *Campylobacter* contamination of chicken skin by application of lytic bacteriophages. *Applied and Environmental Microbiology*, 69(8), 5032-5036.
- Granger, C. B., McMurray, J. J., Yusuf, S., Held, P., Michelson, E. L., Olofsson, B. & CHARM Investigators and Committees. (2003). Effects of candesartan in patients with chronic heart failure and reduced left-ventricular systolic function intolerant to angiotensin-converting-enzyme inhibitors: the CHARM-Alternative trial. *The Lancet*, 362(9386), 772-776.
- Guerra, M. M. M., Almeida, A. M., & Willingham, A. L. (2016). An overview of food safety and bacterial foodborne zoonoses in food production animals in the Caribbean region. *Tropical animal health and production*, 1-14.
- Hagiwara, S. Y., Takahashi, M., Shen, Y., Kaihou, S., Tomiyama, T., Yazawa, M., ... & Terazawa, M. (2005). A phytochemical in the edible Tamogi-take mushroom (*Pleurotus cornucopiae*), D-mannitol, inhibits ACE activity and lowers the blood pressure of spontaneously hypertensive rats. *Bioscience, biotechnology, and biochemistry*, 69(8), 1603-1605.
- Hajieh, G. S., Mohammad, J., Ahad, H., Tahmineh, N., Ali, S., & Ebrahim, R. (2016). The prevalence of bacterial contamination of table eggs from retails markets by *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter jejuni* and *Escherichia coli* in Shahrekord, Iran. *Jundishapur Journal of Microbiology*, 2011(4, Autumn), 0-0.
- Hamrick, K. (2016). USDA Economic Research Service-Background.

- Hansen, K., Adersen, A., Smitt, U. W., Nyman, U., Christensen, S. B., Schwartzner, C., & Wagner, H. (1996). Angiotensin converting enzyme (ACE) inhibitory flavonoids from *Erythroxylum laurifolium*. *Phytomedicine*, 2(4), 313-317.
- Harrison, W. A., Griffith, C. J., Tennant, D., & Peters, A. C. (2001). Incidence of *Campylobacter* and *Salmonella* isolated from retail chicken and associated packaging in South Wales. *Letters in Applied Microbiology*, 33(6), 450-454.
- Hernández-Ledesma, B., Del Mar Contreras, M., & Recio, I. (2011). Antihypertensive peptides: Production, bioavailability and incorporation into foods. *Advances in Colloid and Interface Science*, 165(1), 23-35.
- Heyndrickx, M., Vandekerchove, D., Herman, L., Rollier, I., Grijspeerdt, K., & De Zutter, L. (2002). Routes for *Salmonella* contamination of poultry meat: epidemiological study from hatchery to slaughterhouse. *Epidemiology and Infection*, 129(02), 253-265.
- Hobb, R. I., Fields, J. A., Burns, C. M., & Thompson, S. A. (2009). Evaluation of procedures for outer membrane isolation from *Campylobacter jejuni*. *Microbiology*, 155(3), 979-988.
- Hoffmann, S., Maculloch, B., & Batz, M. (2015). Economic burden of major foodborne illnesses acquired in the United States. *Econ Res Serv*, US Dep Agric, Washington, DC), pp EIB-140.
- Hofstad, M., John, B., Calnek, B., Reid, W., Yoder, H. (1992). Diseases of Poultry. 8th edition. *Panama Education Book Agency*, New Delhi, Ind, 65-123.
- Hong, F., Ming, L., Yi, S., Zhanxia, L., Yongquan, W., & Chi, L. (2008). The antihypertensive effect of peptides: A novel alternative to drugs? *Peptides*, 29: 1062-1071.
- Hulin, V., Mathot, A. G., Mafart, P., & Dufosse, L. (1998). Antimicrobial properties of essential oils and flavour compounds. *Sciences des Aliments* (France).
- Humphery, T. J., Henley, A., & Lanning, D. G. (1993). The colonization of broiler chickens with *Campylobacter jejuni*: some epidemiological investigations. *Epidemiology and Infection*, 110(03), 601-607.
- Hong, F., Ming, L., Yi, S., Zhanxia, L., Yongquan, W., & Chi, L. (2008). The antihypertensive effect of peptides: A novel alternative to drugs? *Peptides*, 29: 1062-1071.
- ICMSF – International Commission on Microbiological Specification for Foods (2005). *Microorganisms in foods 6* (2nd ed.). New York: Kluwer Academic/Plenum Publishers.
- Jackson, V., Blair, I. S., McDowell, D. A., Kennedy, J., & Bolton, D. J. (2007). The incidence of significant foodborne pathogens in domestic refrigerators. *Food control*, 18(4), 346-351.
- Jacobs-Reitsma, W. (2000). *Campylobacter in the food supply*.

- Je, J. Y., Park, P. J., Kim, B., & Kim, S. K. (2006). Antihypertensive activity of chitin derivatives. *Biopolymers*, 83(3), 250-254.
- John, S., & Schmieder, R. E. (2003). Potential mechanisms of impaired endothelial function in arterial hypertension and hypercholesterolemia. *Current hypertension reports*, 5(3), 199-207.
- Junqueira-Gonçalves, M. P., Yáñez, L., Morales, C., Navarro, M., A Contreras, R., & Zúñiga, G. E. (2015). Isolation and Characterization of Phenolic Compounds and Anthocyanins from Murta (*Ugni molinae* Turcz.) Fruits. Assessment of Antioxidant and Antibacterial Activity. *Molecules*, 20(4), 5698-5713.
- Kadariya, J., Smith, T. C., & Thapaliya, D. (2014). Staphylococcus aureus and staphylococcal food-borne disease: an ongoing challenge in public health. *BioMed research international*.
- Kähkönen, M. P., Hopia, A. I., & Heinonen, M. (2001). Berry phenolics and their antioxidant activity. *Journal of Agricultural and Food Chemistry*, 49(8), 4076-4082.
- Khanal, R. C., Howard, L. R., & Prior, R. L. (2009). Procyanidin composition of selected fruits and fruit byproducts is affected by extraction method and variety. *Journal of agricultural and food chemistry*, 57(19), 8839-8843.
- Khoddami, A., Wilkes, M. A., & Roberts, T. H. (2013). Techniques for analysis of plant phenolic compounds. *Molecules*, 18(2), 2328-2375.
- Kiran, S. R., Devi, P. S., & Reddy, K. J. (2008). Evaluation of in vitro antimicrobial activity of leaf and stem essential oils of *Chloroxylon swietenia* DC. *World Journal of Microbiology and Biotechnology*, 24(9), 1909-1914.
- Kwon, Y. I., Apostolidis, E., & Shetty, K. (2008). In vitro studies of eggplant (*Solanum melongena*) phenolics as inhibitors of key enzymes relevant for type 2 diabetes and hypertension. *Bioresource Technology*, 99(8), 2981-2988.
- Lai, P. K., & Roy, J. (2004). Antimicrobial and chemopreventive properties of herbs and spices. *Current medicinal chemistry*, 11(11), 1451-1460.
- Lassissi, T.A., Hettiarachchy, N.S., Rayaprolu, S.J., Kannan, A., & Davis, M. (2014). Functional properties and Angiotensin-I converting enzyme inhibitory activity of soy-whey proteins and fractions. *Food Research International*, 64: 598-602.
- Lee, D. H., Kim, J. H., Park, J. S., Choi, Y. J., & Lee, J. S. (2004). Isolation and characterization of a novel angiotensin I-converting enzyme inhibitory peptide derived from the edible mushroom *Tricholoma giganteum*. *Peptides*, 25(4), 621-627.
- Lee, S. H., Qian, Z. J., & Kim, S. K. (2010). A novel angiotensin I converting enzyme inhibitory peptide from tuna frame protein hydrolysate and its antihypertensive effect in

- spontaneously hypertensive rats. *Food Chemistry*, 118(1), 96-102.
- Leistner, L. (1995). Principles and applications of hurdle technology. In *New methods of food preservation* (pp. 1-21). Springer US.
- Li, R., Hettiarachchy, N., Rayaprolu, S., Eswaranandam, S., Howe, B., Davis, M., & Jha, A. (2014). Phenolics and Antioxidant Activity of Saskatoon Berry (*Amelanchier alnifolia*) *Pomace Extract*. *Journal of medicinal food*, 17(3), 384-392.
- Lieberman, J. (1975). Elevation of serum angiotension-converting-enzyme (ACE) level in sarcoidosis. *The American journal of medicine*, 59(3), 365-372.
- Liesmaa, I. (2010). Regulation of Kinin Receptor Expression in Heart Failure with a Focus on Vascular Endothelium.
- Liu, D. Z., Lin, Y. S., & Hou, W. C. (2004). Monohydroxamates of aspartic acid and glutamic acid exhibit antioxidant and angiotensin converting enzyme inhibitory activities. *Journal of agricultural and food chemistry*, 52(8), 2386-2390.
- Liu, H., Guo, X., Li, J., Zhu, D., & Li, J. (2013). The effects of MgSO₄, d-glucono- δ -lactone (GDL), sucrose, and urea on gelation properties of pectic polysaccharide from soy hull. *Food Hydrocolloids*, 31(2), 137-145.
- Liu, R. H. (2003). Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *The American journal of clinical nutrition*, 78(3), 517S-520S.
- Lowenthal, J. W., O'Neil, T. E., David, A., Strom, G., & Andrew, M. E. (1999). Cytokine therapy: a natural alternative for disease control. *Veterinary immunology and immunopathology*, 72(1), 183-188.
- Madhavi, D. L., Deshpande, S. S., & Salunkhe, D. K. (1995). Food antioxidants: Technological: Toxicological and health perspectives. *CRC Press*. New York: Marcel Dekker, 490p.
- Malenčić, D., Cvejić, J., & Miladinović, J. (2012). Polyphenol content and antioxidant properties of colored soybean seeds from Central Europe. *Journal of medicinal food*, 15(1), 89-95.
- Malik, S., Wong, N. D., Franklin, S. S., Kamath, T. V., Gilbert, J. L., Pio, J. R., & Williams, G. R. (2004). Impact of the metabolic syndrome on mortality from coronary heart disease, cardiovascular disease, and all causes in United States adults. *Circulation*, 110(10), 1245-1250.
- Mallikarjun Gouda, K. G., Gowda, L. R., Rao, A. A., & Prakash, V. (2006). Angiotensin I-converting enzyme inhibitory peptide derived from glycinin, the 11S globulin of soybean (*Glycine max*). *Journal of Agricultural and Food Chemistry*, 54(13), 4568-4573.

- Maruyama, S., & Suzuki, H. (1982). A peptide inhibitor of angiotensin I converting enzyme in the tryptic hydrolysate of casein. *Agricultural and Biological Chemistry*, 46(5), 1393-1394.
- Mataragas, M., Skandamis, P. N., & Drosinos, E. H. (2008). Risk profiles of pork and poultry meat and risk ratings of various pathogen/product combinations. *International Journal of Food Microbiology*, 126(1), 1-12.
- Mbata, T. I. (2005). Poultry meat pathogens and its control. *Internet J. Food Safety*, 7, 20-28.
- Mead, P. S., Slutsker, L., Dietz, V., McCaig, L. F., Bresee, J. S., Shapiro, C., ... & Tauxe, R. V. (1999). Food-related illness and death in the United States. *Emerging infectious diseases*, 5(5), 607.
- Medic, J., Atkinson, C., & Hurburgh Jr, C. R. (2014). Current knowledge in soybean composition. *Journal of the American Oil Chemists' Society*, 91(3), 363-384.
- Meng, J., & Doyle, M. P. (1998). Emerging and evolving microbial foodborne pathogens. *Bulletin de l'Institut Pasteur*, 96(3), 151-163.
- [Microvet] University of Arizona. 2011. Course MIC419: Infectious diseases. Available from: <http://microvet.arizona.edu/Courses/MIC419/Tutorials/infectiousdisease.html>. Accessed June 2011.
- Moore, J. E., Wilson, T. S., Wareing, D. R., Humphrey, T. J., & Murphy, P. G. (2002). Prevalence of thermophilic *Campylobacter* spp. in ready-to-eat foods and raw poultry in Northern Ireland. *Journal of Food Protection*®, 65(8), 1326-1328.
- Morris, G. K., & Wells, J. G. (1970). *Salmonella* contamination in a poultry-processing plant. *Applied microbiology*, 19(5), 795-799.
- Mozaffarian, D., Benjamin, E. J., Go, A. S., et al. (2016) Heart disease and stroke statistics: a report from the American Heart Association. *Circulation*, 131:e29-322.
- Mozaffarian, D., Benjamin, E. J., Go, A. S., Arnett, D. K., Blaha, M. J., Cushman, M., ... & Howard, V. J. (2016). Executive Summary: Heart Disease and Stroke Statistics-2016 Update: A Report From the American Heart Association. *Circulation*, 133(4), 447.
- Mullally, M.M., Meisel, H., & FitzGerald, R.J. (1996). Synthetic peptides corresponding to α -lactalbumin and β -lactoglobulin sequences with angiotensin-I-converting enzyme inhibitory activity. *Biological Chemistry Hoppe-Seyler*, 377: 259-260.
- Murray-Kolb, L. E., Welch, R., Theil, E. C., & Beard, J. L. (2003). Women with low iron stores absorb iron from soybeans. *The American journal of clinical nutrition*, 77(1), 180-184.

- Nakamura, Y., Watanabe, S., Miyake, N., Kohno, H., & Osawa, T. (2003). Dihydrochalcones: evaluation as novel radical scavenging antioxidants. *Journal of Agricultural and Food Chemistry*, 51(11), 3309-3312.
- Nwankwo, T., Yoon S.S., Burt V., & Gu Q. (2013). Hypertension among adults in the US: National health and nutrition examination survey, 2012-2012. NCHS Data Brief, No. 133. Hyattsville, MD: National Center for Health Statistics, Centers for Disease Control and Prevention, *US Department of Health and Human Services*.
- Oboh, G., Akinyemi, A. J., & Ademiluyi, A. O. (2013). Inhibitory effect of phenolic extract from garlic on angiotensin-1 converting enzyme and cisplatin induced lipid peroxidation-in vitro. *Int J Biomed Sci*, 9(2), 98-106.
- O'Bryan, C. A., Kushwaha, K., Babu, D., Crandall, P. G., Davis, M. L., Chen, P., ... & Ricke, S. C. (2014). Soybean Seed Coats: A Source of Ingredients for Potential Human Health Benefits-A Review of the Literature. *Journal of Food Research*, 3(6), 188.
- Oliveira, I., Sousa, A., Ferreira, I. C., Bento, A., Estevinho, L., & Pereira, J. A. (2008). Total phenols, antioxidant potential and antimicrobial activity of walnut (*Juglans regia* L.) green husks. *Food and chemical toxicology*, 46(7), 2326-2331.
- Oliveira, M. R. R., Quadrado, M. G., & Mastro, N. L. (2007). Analysis of Gamma-irradiated Soybean Components by Electron Paramagnetic Resonance. *Proceedings IJM Cancum, Mexico*.
- Painter, J. A., Hoekstra, R. M., Ayers, T., Tauxe, R. V., Braden, C. R., Angulo, F. J., & Griffin, P. M. (2013). Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. *Emerg Infect Dis*, 19(3), 407-415.
- Pandey, K. B., & Rizvi, S. I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative medicine and cellular longevity*, 2(5), 270-278.
- Park, E. J., & Jhon, D. Y. (2010). The antioxidant, angiotensin converting enzyme inhibition activity, and phenolic compounds of bamboo shoot extracts. *LWT-Food Science and Technology*, 43(4), 655-659.
- Park, S. F. (2002). The physiology of *Campylobacter* species and its relevance to their role as foodborne pathogens. *International journal of food microbiology*, 74(3), 177-188.
- Park, S. H., Choi, M. R., Park, J. W., Park, K. H., Chung, M. S., Ryu, S., & Kang, D. H. (2011). Use of organic acids to inactivate *Escherichia coli* O157: H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* on organic fresh apples and lettuce. *Journal of Food Science*, 76(6), M293-M298.

- Parker, M. (1990). Enteric infections. In Topley and Wilson's principles of bacteriology, virology and immunity. Edited by Parker MT, Collier LH. Edward Arnold, London, 424-446.
- Patrick, M. E., Christiansen, L. E., Wainø, M., Ethelberg, S., Madsen, H., & Wegener, H. C. (2004). Effects of climate on incidence of *Campylobacter* spp. in humans and prevalence in broiler flocks in Denmark. *Applied and environmental microbiology*, 70(12), 7474 - 7480.
- Pereira, J. A., Pereira, A. P., Ferreira, I. C., Valentão, P., Andrade, P. B., Seabra, R. & Bento, A. (2006). Table olives from Portugal: phenolic compounds, antioxidant potential, and antimicrobial activity. *Journal of Agricultural and Food Chemistry*, 54(22), 8425-8431.
- Petersen, K. E., & James, W. O. (1998). Agents, vehicles, and causal inference in bacterial foodborne disease outbreaks: 82 reports (1986-1995). *Journal of the American Veterinary Medical Association*, 212(12), 1874-1881.
- Phanthong, P., Lomarat, P., Chomnawang, M. T., & Bunyaphratharsara, N. (2013). Antibacterial activity of essential oils and their active components from Thai spices against foodborne pathogens. *Science Asia*, 39, 472-476.
- Phommalath, S., Teraishi, M., Yoshikawa, T., Saito, H., Tsukiyama, T., Nakazaki, T., ... & Okumoto, Y. (2014). Wide genetic variation in phenolic compound content of seed coats among black soybean cultivars. *Breeding science*, 64(4), 409.
- Piskernik, S., Klančnik, A., Riedel, C. T., Brøndsted, L., & Možina, S. S. (2011). Reduction of *Campylobacter jejuni* by natural antimicrobials in chicken meat-related conditions. *Food Control*, 22(5), 718-724.
- Pratt, D. E., & Hudson, B. J. (1990). Natural antioxidants not exploited commercially. In Food antioxidants (pp. 171-191). Springer Netherlands.
- Proestos, C., Boziaris, I. S., Nychas, G. J., & Komaitis, M. (2006). Analysis of flavonoids and phenolic acids in Greek aromatic plants: Investigation of their antioxidant capacity and antimicrobial activity. *Food Chemistry*, 95(4), 664-671.
- Proestos, C., Chorianopoulos, N., Nychas, G. J., & Komaitis, M. (2005). RP-HPLC analysis of the phenolic compounds of plant extracts. Investigation of their antioxidant capacity and antimicrobial activity. *Journal of Agricultural and Food Chemistry*, 53(4), 1190-1195.
- Puupponen-Pimiä, R., Nohynek, L., Alakomi, H. L., & Oksman-Caldentey, K. M. (2005). Bioactive berry compounds—novel tools against human pathogens. *Applied Microbiology and Biotechnology*, 67(1), 8-18.
- Ranilla, L. G., Kwon, Y. I., Apostolidis, E., & Shetty, K. (2010). Phenolic compounds, antioxidant activity and in vitro inhibitory potential against key enzymes relevant for

hyperglycemia and hypertension of commonly used medicinal plants, herbs and spices in Latin America. *Bioresource technology*, 101(12), 4676-4689.

- Rauha, J. P., Remes, S., Heinonen, M., Hopia, A., Kähkönen, M., Kujala, T. & Vuorela, P. (2000). Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. *International journal of food microbiology*, 56(1), 3-12.
- Roger, V. L., Go, A. S., Lloyd-Jones, D. M., Adams, R. J., Berry, J. D., Brown, T. M. & Fox, C. S. (2011). Heart disease and stroke statistics—2011 update a report from the American Heart Association. *Circulation*, 123(4), e18-e209.
- Saliu, J. A., Ademiluyi, A. O., Akinyemi, A. J., & Oboh, G. (2012). In vitro antidiabetes and antihypertension properties of phenolic extracts from bitter leaf (*Vernonia amygdalina* Del.). *Journal of Food Biochemistry*, 36(5), 569-576.
- Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M. A., Roy, S. L., ... & Griffin, P. M. (2011). Foodborne illness acquired in the United States—major pathogens. *Emerg Infect Dis*, 17(1).
- Sebei, K., Gnouma, A., Herchi, W., Sakouhi, F., & Boukhchina, S. (2013). Lipids, proteins, phenolic composition, antioxidant and antibacterial activities of seeds of peanuts (*Arachis hypogaea* l) cultivated in Tunisia. *Biological research*, 46(3), 257-263.
- Segura-Campos, M.R., Peralta-González, F., Castellanos-Ruelas, A., Chel-Guerrero, L.A., & Betancur-Ancona, D.A. (2013). Effect of *Jatropha curcas* peptide fraction on the angiotensin I-converting enzyme inhibitory activity. *BioMeb Research International*, d
- Sessa, D. J., & Wolf, W. J. (2001). Bowman–Birk inhibitors in soybean seed coats. *Industrial crops and products*, 14(1), 73-83.
- Shi, J., Nawaz, H., Pohorly, J., Mittal, G., Kakuda, Y., & Jiang, Y. (2005). Extraction of polyphenolics from plant material for functional foods—engineering and technology. *Food reviews international*, 21(1), 139-166.
- Shi, L., Mao, C., Xu, Z., & Zhang, L. (2010). Angiotensin-converting enzymes and drug discovery in cardiovascular diseases. *Drug discovery today*, 15(9), 332-341.
- Silagyi, K., Kim, S. H., Lo, Y. M., & Wei, C. I. (2009). Production of biofilm and quorum sensing by *Escherichia coli* O157: H7 and its transfer from contact surfaces to meat, poultry, ready-to-eat deli, and produce products. *Food microbiology*, 26(5), 514-519.
- Silhavy, T. J., Kahne, D., & Walker, S. (2010). The bacterial cell envelope. *Cold Spring Harbor perspectives in biology*, 2(5), a000414.

- Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture*, 16(3), 144-158.
- Skeggs, L. T., Kahn, J. R., & Shumway, N. P. (1956). The preparation and function of the hypertensin-converting enzyme. *The Journal of experimental medicine*, 103(3), 295-299.
- Smith, T. W., Morgan, J. P., Bakris, G. L., & Sheridan, A. M. (2015). Differences between angiotensin converting enzyme inhibitors and receptor blockers. UpToDate. <http://www.uptodate.com/contents/differences-between-angiotensin-converting-enzyme-inhibitors-and-receptor-blockers>. Accessed December, 29.
- Soybean Production in Arkansas. (2014). Available from: <http://www.uaex.edu/farm-ranch/crops-commercial-horticulture/soybean/>
- Stahl, M., Butcher, J., & Stintzi, A. (2012). Nutrient acquisition and metabolism by *Campylobacter jejuni*. *Front Cell Infect Microbiol*, 2(5).
- Sukumaran, A. T., Nannapaneni, R., Kiess, A., & Sharma, C. S. (2015). Reduction of *Salmonella* on chicken meat and chicken skin by combined or sequential application of lytic bacteriophage with chemical antimicrobials. *International journal of food microbiology*, 207, 8-15.
- Suppakul, P., Miltz, J., Sonneveld, K., & Bigger, S. W. (2003). Antimicrobial properties of basil and its possible application in food packaging. *Journal of agricultural and food chemistry*, 51(11), 3197-3207.
- Sweitzer, N. K. (2003). What is an angiotensin converting enzyme inhibitor? *Circulation*, 108(3), e16-e18.
- Szabo, M. R., Radu, D., Gavrilas, S., Chambre, D., & Iditoiu, C. (2010). Antioxidant and antimicrobial properties of selected spice extracts. *International Journal of Food Properties*, 13(3), 535-545.
- Tapiero, H., Tew, K.D., Ba, G.N., & Mathé, G. (2002). Polyphenols: do they play a role in the prevention of human pathologies? *Biomed Pharmacother*. 56(4):200-7.
- Tauxe, R. V. (1997). Emerging foodborne diseases: an evolving public health challenge. *Emerging infectious diseases*, 3(4), 425.
- Todd, E. C. (1996). Epidemiology of foodborne diseases: a worldwide review. World health statistics quarterly. *Rapport trimestriel de statistiques sanitaires mondiales*, 50(1-2), 30-50.
- Todd, J. J., & Vodkin, L. O. (1993). Pigmented soybean (*Glycine max*) seed coats accumulate proanthocyanidins during development. *Plant Physiology*, 102(2), 663-670.

- Touch, V., Hayakawa, S., Yamada, S., & Kaneko, S. (2004). Effects of a lactoperoxidase–thiocyanate–hydrogen peroxide system on *Salmonella* enteritidis in animal or vegetable foods. *International Journal of Food Microbiology*, 93(2), 175-183.
- Torres, N., Torre-Villalvazo, I., & Tovar, A. R. (2006). Regulation of lipid metabolism by soy protein and its implication in diseases mediated by lipid disorders. *The Journal of nutritional biochemistry*, 17(6), 365-373.
- Troxell, B., Petri, N., Daron, C., Pereira, R., Mendoza, M., Hassan, H. M., & Koci, M. D. (2015). Poultry body temperature contributes to invasion control through reduced expression of *Salmonella* pathogenicity island 1 genes in *Salmonella* enterica Serovars Typhimurium and Enteritidis. *Applied and environmental microbiology*, 81(23), 8192-8201.
- U.S. Department of Agriculture Food Safety and Inspection Service. (2011). Basics for Handling Food Safely. Available at:
http://www.fsis.usda.gov/shared/PDF/Basics_for_Safe_Food_Handling.pdf
- Velayudhan, J., & Kelly, D. J. (2002). Analysis of gluconeogenic and anaplerotic enzymes in *Campylobacter jejuni*: an essential role for phosphoenolpyruvate carboxykinase. *Microbiology*, 148(3), 685-694.
- Villalobos, M. D. C., Serradilla, M. J., Martín, A., Ordiales, E., Ruiz-Moyano, S., & Córdoba, M. D. G. (2015). Antioxidant and antimicrobial activity of natural phenolic extract from defatted soybean flour by-product for stone fruit postharvest application. *Journal of the Science of Food and Agriculture*.
- Villar, A., Paya, M., & Terencio, M. C. (1986). Plants with antihypertensive action. *Fitoterapia*, 52, 131-145.
- Wales, A. D., & Davies, R. H. (2011). A critical review of *Salmonella* Typhimurium infection in laying hens. *Avian Pathology*, 40(5), 429-436.
- Wang, Y., Chen, Q., Cui, S., Xu, X., Zhu, J., Luo, H., ... & Li, F. (2014). Enumeration and characterization of *Salmonella* isolates from retail chicken carcasses in Beijing, China. *Foodborne pathogens and disease*, 11(2), 126-132.
- Whyte, P., Collins, J. D., McGill, K., Monahan, C., & O'mahony, H. (2001). Quantitative investigation of the effects of chemical decontamination procedures on the microbiological status of broiler carcasses during processing. *Journal of Food Protection®*, 64(2), 179-183.
- Wijesekara, I., & Kim, S. K. (2010). Angiotensin-I-converting enzyme (ACE) inhibitors from marine resources: prospects in the pharmaceutical industry. *Marine Drugs*, 8(4), 1080-1093.

- Wilson, D. J., Gabriel, E., Leatherbarrow, A. J., Cheesbrough, J., Gee, S., Bolton, E., & Diggle, P. J. (2008). Tracing the source of campylobacteriosis.
- Winfield, M. D., & Groisman, E. A. (2004). Phenotypic differences between *Salmonella* and *Escherichia coli* resulting from the disparate regulation of homologous genes. *Proceedings of the National Academy of Sciences of the United States of America*, 101(49), 17162-17167.
- Wu, J., Lau, E. H., Xing, Q., Zou, L., Zhang, H., Yen, H. L., ... & Cowling, B. J. (2015). Seasonality of avian influenza A (H7N9) activity and risk of human A (H7N9) infections from live poultry markets. *The Journal of infection*, 71(6), 690.
- Xu, B. J., & Chang, S. K. C. (2007). A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. *Journal of food science*, 72(2), S159- S166.
- Xu, B., & Chang, S. K. (2008). Antioxidant capacity of seed coat, dehulled bean, and whole black soybeans in relation to their distributions of total phenolics, phenolic acids, anthocyanins, and isoflavones. *Journal of agricultural and food chemistry*, 56(18), 8365-8373.
- Yang, B., Yang, H., Li, J., Li, Z., Jiang, Y. (2011). Amino acid composition, molecular weight distribution and antioxidant activity of protein hydrolysates of soy sauce lees. *Journal of Food Chemistry*, 124: 551-555.
- Yang, H., Li, Y., & Johnson, M. G. (2001). Survival and death of *Salmonella* Typhimurium and *Campylobacter jejuni* in processing water and on chicken skin during poultry scalding and chilling. *Journal of Food Protection*®, 64(6), 770-776.
- Yang, Y., Iji, P. A., & Choct, M. (2009). Dietary modulation of gut microflora in broiler chickens: a review of the role of six kinds of alternatives to in-feed antibiotics. *World's Poultry Science Journal*, 65(01), 97-114.
- Zhang, L., Singh, P., Lee, H. C., & Kang, I. (2013). Effect of hot water spray on broiler carcasses for reduction of loosely attached, intermediately attached, and tightly attached pathogenic (*Salmonella* and *Campylobacter*) and mesophilic aerobic bacteria. *Poultry science*, 92(3), 804-810.
- Zhao, C., Ge, B., De Villena, J., Sudler, R., Yeh, E., Zhao, S. & Meng, J. (2001). Prevalence of *Campylobacter* spp., *Escherichia coli*, and *Salmonella* serovars in retail chicken, turkey, pork, and beef from the Greater Washington, DC, area. *Applied and Environmental Microbiology*, 67(12), 5431-5436.

Žilić, S., Akilloğlu, H. G., Serpen, A., Perić, V., & Gökmen, V. (2013). Comparisons of phenolic compounds, isoflavones, antioxidant capacity and oxidative enzymes in yellow and black soybeans seed coat and dehulled bean. *European Food Research and Technology*, 237(3), 409-418.