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Antioxidant properties and anti-cancer effects of polyphenols in sweetpotato leaves

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

by

Josue Bolanos
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Bachelor of Science in Biochemistry, 2018

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Abstract

Although the root is widely consumed, sweetpotato leaves (SPL) are often discarded and are only consumed in a few countries. In the United States, SPL consumption is limited to private gardens, particularly in the Southeastern United States. Not only are SPL a good source of nutrients such as vitamins and minerals, but they also contain polyphenol compounds including the caffeoylquinic acid derivatives and carotenoids such as lutein. Several studies have shown the polyphenol contents and antioxidant capacities of SPL, which vary based on year and variety, while few studies have shown anti-colon cancer effects of SPL. Therefore, this study investigated the impact of year and variety on the antioxidant capacity and total polyphenol content of SPL and the *in vitro* anti-colon cancer effects of SPL on cell viability and apoptosis in Caco-2 cells. The results showed that the 2019 SPL possessed higher total polyphenols and antioxidant capacity compared to the 2018 SPL. Among 2019 SPL varieties, SPL1, SPL3, SPL9, SPL11, and SPL28 were selected to evaluate further anti-colon effects. In these 5 SPL varieties with the highest antioxidant and polyphenol amounts, the major polyphenols, 3,5-dicaffeoylquinic acid, 3,4-dicaffeoylquinic acid, and chlorogenic acid, were identified and quantified. All 5 samples significantly decreased cell viability in a dose-dependent manner and significantly increased apoptosis at 600 μg/mL ($p < 0.05$). Polyphenols and carotenoids are known to be bioactive compounds with potential health-promoting properties. The findings of the present study support that SPLs contain anti-colon cancer properties, and further research is needed.

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Introduction

As our world population is expected to explode to 8.5 billion in 2030 and 9.7 billion in 2050 (United Nations 2019), processes to develop sustainable and effective food systems are in high demand. The sweetpotato plant can provide a solution by reducing waste through by-product utilization. According to the UN's Food & Agricultural Organization (FAO), China produced over 53 million tons of sweetpotato plant in 2018, while all the least developed countries combined produced only about 20 million tons (FAO Statistics 2018). In comparison, the U.S. produced almost 1.8 million tons in 2019 (USDA 2019). Although the root is widely consumed, about 95-98% of the sweetpotato leaves in China are discarded or used as animal feed (Hue *et al.,* 2012). In the United States, sweetpotato leaf consumption is limited. However, the plant is produced commercially and in private gardens, particularly in the Southeastern United States (Johnson and Pace 2010).

The wasted leaves could not only provide sustenance for an exploding population but contain health-promoting properties that are not well investigated. These abilities come from their bioactive compounds, namely their high levels of polyphenols common in leafy green vegetables. Polyphenols are characterized by multiple phenol rings, with a varied amount of hydroxyl groups bound to each ring. Their antioxidant potential comes from a propensity of a homolytic cleavage and donation of the hydroxyl group electrons to free radical compounds. Therefore, polyphenols decrease oxidation in plant cells, normally caused by stress, including sunlight radiation and plant senescence processes. When consumed by humans, the protective characteristics of polyphenols are absorbed and used by human cells. However, the bioavailability and bioaccessibility of polyphenols in human absorption is complex, variable, and lacks human research trials.

The polyphenols in sweetpotato leaves fight oxidation in human cells, and oxidation is an agent of many non-communicable diseases, including heart disease, diabetes, and cancer. Globally, cancer ranks as the number two cause of death, and colon cancer ranks as the fourth cancer in the number of fatalities (Favoriti *et al.,* 2016)**.** However, few original research papers have investigated the link between sweetpotato leaf polyphenols and proliferation of colon cancer (Taira *et al.,* 2014; Vishnu *et al.,* 2019; Kurata *et al.,* 2007,). Therefore, to better understand the potential health-promoting activities of sweetpotato leaves, this research drives to characterize and compare the antioxidant and anti-colon cancer properties in different varieties and production years of sweetpotato leaves.

CHAPTER 1: Literature Review

1. Colorectal Cancer

Colorectal cancer (CRC) is cancer found either in the colon or rectum. Colon cancer begins as benign clumps of cells (polyps) which produce few if any, symptoms until the cancer progresses. The rectum starts at the final segment of the colon down to the anus. While both rectal and colon cancers are similar, rectal cancer is more difficult to remove (Feldman *et al.,* 2020). CRC is also known as adenocarcinoma, and it arises from epithelial glands of the large intestine when the gland cells mutate. Then, the cells proliferate to give rise to a benign adenoma which then can undergo malignant transformation into a carcinoma and mutate over decades (Rawla *et al.,* 2019).

Colorectal cancer is a leading cause of mortality and morbidity in the world (Favoriti *et al.* 2016). It is the third most common malignancy and the fourth leading cause of cancer-related deaths worldwide, annually accounting for approximately 1,400,000 new cases and about 700,000 deaths worldwide (Favoriti *et al.* 2016). According to the 2018 Global Cancer Observatory (GLOBOCAN) data, colon cancer specifically is the fourth most incident worldwide, while rectal cancer is the eighth most incident. Colon and rectal cancer together is the third most commonly diagnosed form of cancer in the world, making up 11% of all annual cancer diagnoses (Rawla *et al.,* 2019).

CRC has a higher incidence in men than women and is 3-4 times more common in developed than in developing nations. For colon cancer, Southern Europe, Northern Europe, and Australia/New Zealand are the regions of highest incidence. For rectal cancer, Eastern Europe, Australia/New Zealand, and Eastern Asia are regions of highest incidence. North America also has one of the highest incidence rates for both cancers. On the other hand, all regions of Africa

and Southern Asia have the lowest incidence rates for both cancers and both sexes (Rawla *et al.,* 2019). CRC incidence varies between developed and developing nations, which can be explained by the causal relationship of increasing human development index (HDI) (include life expectancy, literary, education, GDP, etc) with increasing incidence (Rawla *et al.,* 2019).

Although rates have been decreasing for those over 50 years of age in the U.S. over the past decades, the age group between 20-49 years have seen a growing incidence (9.3 per 100,000 in 1975 to 13.7 per 100,000 in 2015) (Rawla *et al.,* 2019). By the year 2030, CRC is expected to increase to over 2.2 million annual new cases and 1.1 million annual deaths, due to the economic development of less-developed nations. Economic development brings environmental and behavioral changes, including a switch to sedentary lifestyles, higher obesity, and higher consumption of processed foods (Rawla *et al.,* 2019).

Fortunately, a diet of fruits and vegetables protects against CRC due to their antioxidants, vitamins, fiber, and folic acid. Moreover, dietary fiber plays a part in cutting CRC pathogenesis. Fiber reduces CRC incidence by lowering colon pH, reducing colon transit time, and increasing folic acid and vegetable absorption (Gandomani *et al.,* 2017).

In Vitro Colon Cancer Cell Model

To investigate the effects of dietary bioactive compounds on colon cancer, *in vitro* studies can be performed using colon cancer cell lines. Among the available colon cancer cell lines are DLD-1, HT-29, and Caco-2. Derived from a colon carcinoma, Caco-2 cells have advantageous properties in their ability to differentiate into a cell monolayer with qualities of absorptive enterocytes, such as a brush border layer and expression of enzymes and transporter proteins (Verhoeckx *et al.,* 2015). It has been found that Caco-2 monolayers cultured under suitable

conditions (such as with permeable filters) correlate with studies on the absorption of bioactive compounds after oral intake in humans (Verhoeckx *et al.,* 2015).

Mechanism of Cancer Cell Death

In general, cancer cell death can be classified as either necrosis or apoptosis based on biochemical and morphological characteristics. Necrosis is the unprogrammed, or accidental, death of cells and living tissues. During necrosis, ion permeability of the plasma membrane increases, followed by cell swelling, and final plasma membrane rupture within minutes. This rupturing process is called osmotic lysis (Zhang *et al.,* 2018)

On the other hand, apoptosis is programmed cell death. It is the most common form of eukaryotic cell death, happening throughout the body. It occurs in the erasure of autoreactive T cells during maturation of the thymus gland, in the senescence of neutrophil polymorphs (most abundant white blood cell type), in the removal of growth factors like IL-2, or reaction to physiological stimuli like tumor necrosis factor and glucocorticoids. Cytotoxic T lymphocytes, natural killer (NK) cells, ionizing radiation, and monoclonal antibodies (e.g. anti-Fas and anti-APO-1) are all apoptosis inductors (Bunel *et al.,* 2013).

In apoptosis, the cell's membrane undergoes blebbing (zeiosis). A bleb is a bulge of the plasma membrane of the cell. Subsequently, the cell's membrane condenses, and endogenous endonucleases are activated. The endonuclease, which is ion-dependent (Ca^{+2} or Mg^{+2}), cleaves double-stranded DNA at the internucleosomal linker region, which is the easiest to reach. The cleaving results in mono- and oligonucleosomes. The nucleosomes cannot be cleaved further by the endonucleases because the DNA of the nucleosomes is tightly bound to the core histones

(H2A, H2B, H3, and H4). Histones are proteins in the cell nuclei of eukaryotes that package and organize DNA into the nucleosomes. (Bunel *et al*., 2013; Zhang *et al.,* 2018).

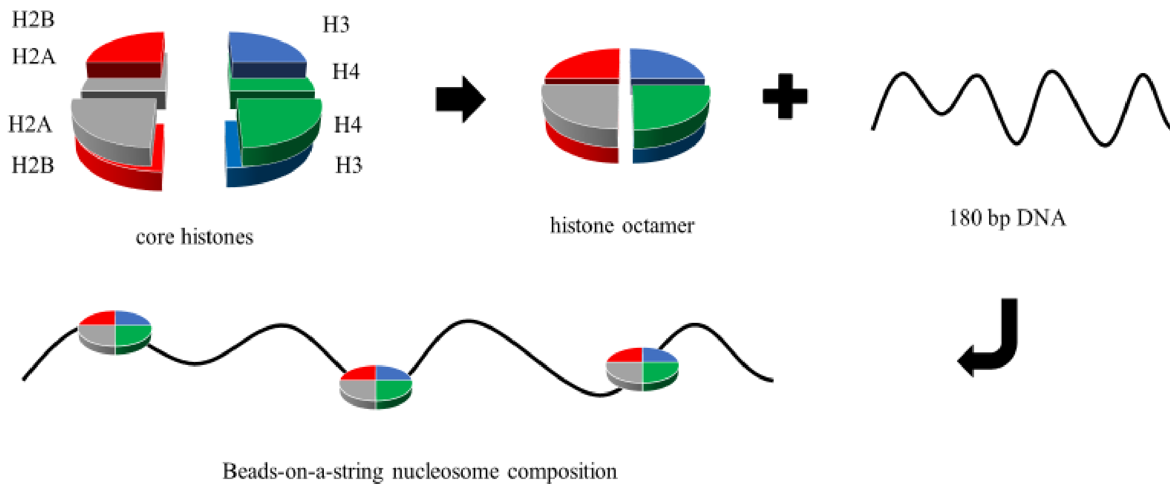

Figure 1. Formation of nucleosomes composed of histone octamers and DNA (modified from Morgan, 2007)

The mono- and oligonucleosomes produced are separate pieces of a 180 base pair which can be revealed in an agarose gel as a “DNA ladder” after extraction and separation of the nucleosomal fragments. The nucleosomal enrichment of the cytoplasm is only possible because the DNA degradation of apoptotic cells occurs before (several hours) the outer plasma membrane breaks down (Zhang *et al.,* 2018).

2. Sweetpotato Plant

Ipomoea batatas, or sweetpotato, is a widely popular plant, belonging to the morning glory family, *Convolvulaceae.* Although the family houses more than 1,000 species, the genera *Ipomoea* only includes the most used species, namely *batatas, aquatica* and a few flowering species, the morning glories.

2.1 General Overview

Background

Historically, *Ipomoea batatas* are native to the tropical regions in the Americas, where domestication of sweetpotato occurred in either Central or South America. In Central America, domesticated sweetpotatoes were present at least 5,000 years ago, with their origin happening somewhere between Mexico and Venezuela. Domesticated sweetpotatoes were most likely spread by local people to the Caribbean and South America by 2500 BCE. Europeans translocated the crop from its native home in the Americas to Africa and India by the early 1500s, China by 1594, and Japan by 1597. Furthermore, sweetpotato ranks seventh among food crops worldwide, with an annual production of 115 million metric tons (Mohanraj and Sivasankar, 2014). Worldwide adoption of *I. batatas* is due to its ecological versatility and hardiness.

Moreover, the sweetpotato tops can be continuously harvested over many months, not just once like many other commercial vegetables, producing better yields (Yoshimoto *et al.,* 2002). More than one hundred million tons of sweetpotato plants are produced annually, 92% of which are produced in Asia and the Pacific Islands: 89% of which is grown in China (Mohanraj and Sivasankar, 2014).

Environmental Conditions

I. batatas are not marked as invasive or dangerous crop, but it does grow rapidly and in a wide range of environmental conditions. Annual rainfalls of 7.5 - 10 decimeters (dm) are most suitable, with a minimum of 5.0 dm in the growing season. The crop is sensitive to drought at the tuber initiation stage, which is 50 - 60 days after planting. Furthermore, waterlogging must be

avoided as it causes tuber rots and reduces growth. They grow best in temperatures above 24 °C, and a pH of 4.5 to 7 in mostly tropical and sub-tropical climates (Abidin, 2004).

Consumption

It has been reported that fresh young leaves are consumed as fresh vegetable in West Africa and Asia, particularly in Taiwan and China (Karna *et al.,* 2011). However, in China, the number one producer, over 95% are discarded except for a small percentage used as livestock feed, resulting in a waste of natural resources (Huang *et al.,* 2013). On the other hand, countries throughout West Africa and Southeast Asia exemplify sustainable practices by utilizing the discarded leaves (Koncic *et al.,* 2012). In the U.S., sweetpotato leaf consumption is limited (Johnson and Pace 2010). However, it was reported that sweetpotato leaves are consumed as a vegetable by African Americans in the Southeastern United States (Huang *et al.,* 2007).

2.2 Nutrients in Sweetpotato Leaves (SPL)

SPL Moisture Content

According to a research article, moisture content of sweetpotato leaves in forty cultivars ranged from 84.1 - 88.9 g/100 g fresh weight (84-90% moisture content) (Sun et al., 2014). More literature reports similar moisture contents of SPL from 80% to 90% (Ishida et al., 2000). Variation in moisture content can be influenced by the maturity of the sweetpotato leaves (Sun et al., 2014).

SPL Protein Content

I. batatas leaves contain protein and a good amino acid score (Koncic et al., 2012). “Score” refers to the PDCAAS (Protein Digestibility-Corrected Amino Acid Score). It is a rating adopted

by the FDA and FAO as the preferred method of protein quality determination. (Boutrif, 1991). In support, crude protein content in a study ranged from 16.68-31.08 g / 100 g of dry weight (DW) in forty cultivars (Sun et al., 2014). Another study analyzed two cultivars from Japan and found them to be 29.5 and 24.5 g / 100 g DW, in range with Sun et al (2014) values. In fresh weight, the value is converted to about 3 g / 100 g DW, ranking higher than the roots (1.28-2.13 g / 100 g fresh weight) and similar to that of milk (3.3 g / 100 g fresh weight) (Ishida et al. 2000).

SPL Fiber, Vitamins, and Minerals

SPLs are rich in soluble dietary fiber and have high mineral content, particularly iron, and have high vitamin content, such as vitamin B2, vitamin C, and vitamin E (Koncic et al., 2012; Abidin, 2004). Several factors are responsible for total crude fiber content such as genotype, maturity, and nutritional composition (Sun et al., 2014).

Islam (2006) characterized the average contents of minerals and vitamins in their developed 'Suioh' cultivar. These were 117 mg calcium, 1.8 mg iron, 7.2 mg vitamin C, 1.6 mg vitamin E, and 0.56 mg vitamin K / 100 g fresh weight (FW) of leaves. The calcium and vitamin E levels rank higher than sweetpotato tubers, which have 0.69 mg iron, 26 mg calcium, and 1.4 mg vitamin E / 100 g FW (USDA 2019).

2.3 Phytochemicals in SPL

Anthocyanins and phenolic acids fall into a major group of phytochemicals called polyphenols, which are the bioactive compounds found in sweetpotato leaves. Phytochemicals in foods are bioactive, meaning they can modulate metabolic processes resulting in the promotion of better health. Phytochemicals have many beneficial roles: antioxidant activity,

inhibition/induction of enzymes, modulation of receptor activities, and inhibition/induction of gene expression (Galanakis, 2017).

Furthermore, the bioavailability and bioaccessibility of each bioactive compound differ, and the most abundant compounds in ingested fruit or vegetables do not necessarily convert to be the highest amounts of active metabolites in target tissues (Galanakis, 2017). Thus, this is important when studying the specific health effects of the bioactive compounds of SPL because bioactivity does not necessarily correlate with bio-accessibility. Studies that analyze bio-accessibility through postprandial measurements of tissue metabolites are of great importance to understand the true health effects of phytochemicals. Phytochemicals include extremely varied classes including polyphenols, carotenoids, phytosterols, organosulfur compounds, and tocopherols (Galanakis, 2017). Groups differ in structure and function, and in what foods they exist. Also, they differ in concentration in their respective foods, absorption in the human body, and their antioxidant activities.

Although the primary bioactive compounds that SPLs contain are polyphenols, SPLs also contain the carotenoids β-carotene and lutein. Lutein is a functional component in humans, playing an important role in the prevention of age-related macular degeneration (Abidin, 2004).

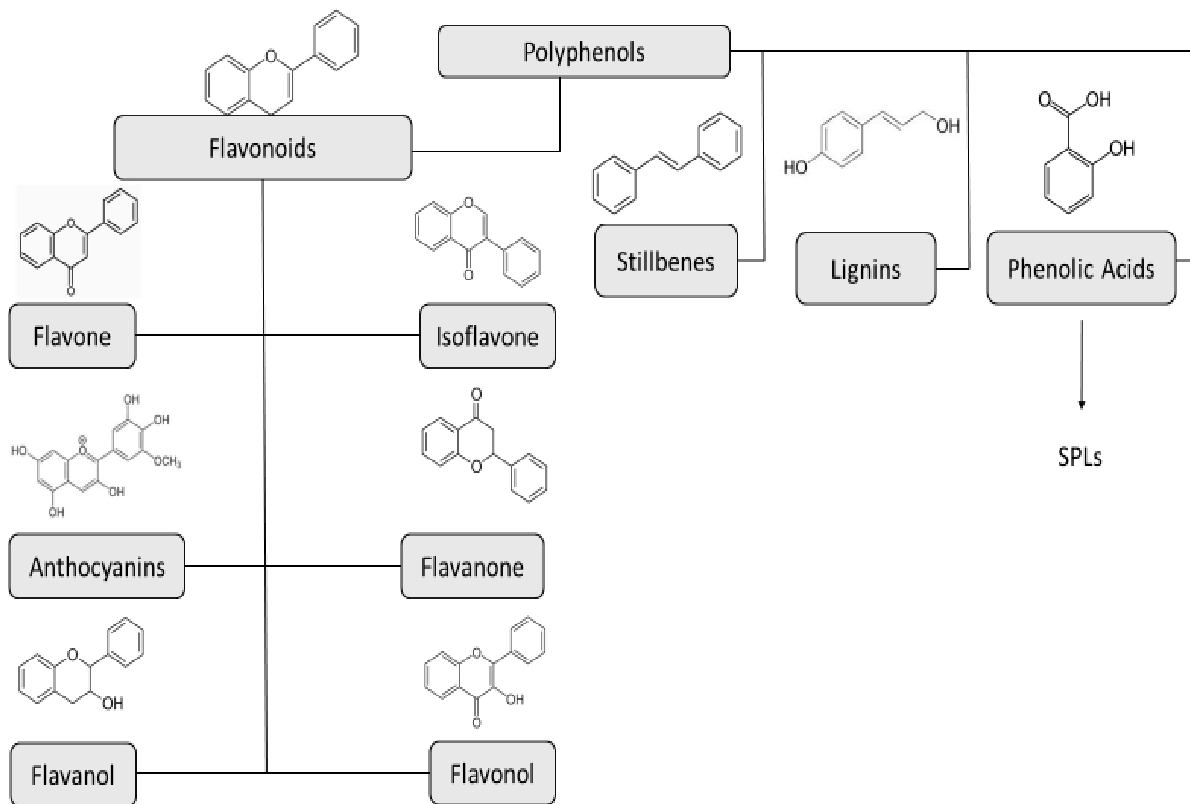

Figure 2. Classification of polyphenols including flavonoid and non-flavonoid groups (modified from Arora et al., 2019)

As seen in Figure 1, polyphenols are a class of phytochemicals that include flavonoids, lignans, stilbenes, and phenolic acids. Phenolic acids are classified as a major, diverse group of polyphenols found in many plants. They function in supporting plant structure and metabolism. Phenolic acids are found in vegetables and fruits, and function as antioxidants by protecting the human body from oxidative stress, a catalyst for cancer, aging, and cardiovascular maladies (Galanakis, 2017).

The flavonoid subclass itself includes more subclasses, with slight structural variance. Anthocyanins are the main group of easily visible polyphenols as they make up the red and purple colors of many plants, including purple variations of sweetpotato leaves. They include many metabolites, which function as key antioxidants and food colorants (Islam, 2006).

Flavones, from flavus meaning yellow, are a subclass of flavonoid which share a 2-phenylchromen-4-one backbone. They are found in spices, fruits, and vegetables. Common flavones include apigenin, luteolin, and tangeretin. Flavones are one of the components of I. batatas leaf (Zhao et al. 2007; Ojong et al., 2008). Flavones have several physiological effects such as immunomodulating activity, antioxidant properties, and hypolipemic and hypoglycemic effects (Zhao et al., 2007). The study of flavonoids has been intensive due to their antioxidant properties that improve health. Their antioxidant mechanisms are defined by the kind of radical they scavenge. After extraction of flavonoids from SPL, one study not only measured general radical scavenging activity by DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) radical scavenging assay but also used superoxide anion radical scavenging activity assay and hydroxyl radical scavenging activity assay (Huang et al., 2013).

Polyphenols

Polyphenols in sweetpotato leaves are primarily phenolic acids, specifically caffeic acid (CA) and 5-caffeoylquinic acid (CQA) derivatives. The derivatives include chlorogenic acid, 3,4-diCQA, 3,5-diCQA, 4,5-diCQA, and 3,4,5-triCQA. In a study, all CQA derivatives (not including caffeic acid) were positively correlated with total polyphenol contents of sweetpotato leaves (Islam et al., 2002). This correlation implicates that CQA derivatives make up much of the total sweetpotato leaf polyphenol content. The study reported that the highest polyphenol concentration was in the leaves (6.19 g ± 0.14 g /100 g DW), followed by petioles (leafstalk) (2.97 g ± 0.26 g/100 g DW), stems (1.88 g ± 0.19 g /100 g DW), and finally roots (<1.00 g / 100 g DW) (Islam et al., 2002). This showed that polyphenol concentrations in sweetpotatoes were organ dependent, and the leaves contained the highest amounts. The study also classified and quantified the total leaf polyphenol contents of 1389 genotypes (Islam et al., 2002). This large

sample size was grown in 2000 and 2001, with a small interannual variation. This study informed of genotypic variation of sweetpotato leaves, which ranged from 1.42 g polyphenols / 100 g DW leaves to 17.1 g polyphenols / 100 g DW leaves in the lowest and highest genotypes, respectively (Islam et al., 2002). These SPL had higher total polyphenol content in comparison to the total content of polyphenols in spinach samples, ranging from 0.18 to 0.50 g polyphenols / 100 g of dry matter (Ligor et al., 2013) and in kale samples, where 0.03 g polyphenols / 100 g dry matter were found (Ligor et al., 2013).

Although phenolic acids make up most of the polyphenols in SPL, sweetpotato leaves can also contain flavonoids, namely anthocyanins and flavones. A study measured flavonoid content using catechin, a common standard for flavonoids (Hue et al. 2012). Flavonoid quantities ranged between 96 ± 47.6 µg/g and 263.5 ± 43.5 µg/g (DW.) This range represents the averages of the cultivar with the lowest flavonoid content and the highest flavonoid content. The Batu Biasa cultivar had the highest flavonoid content, followed by Batu Pituh and Oren varieties. Leaves of onion (1497.5 µg/g quercetin, 391.0 µg/g luteolin, and 832.0 µg/g kaempferol) black tea (1491.0 µg/g), and papaya shoots (1264.0 µg/g) contained higher flavonoids contents, but soybean sprouts (78.5 µg/g), red spinach (29.5 µg/g), and kailan (Chinese broccoli) (14.5 µg/g) showed lower flavonoid content compared to I. batatas leaf (Hue et al., 2012; Miean and Mohamed, 2001).

There can be anthocyanins present depending on the color of the leaves since leaf color can be green, yellowish-green, or purple in part or on the entire leaf (Mohanraj and Sivasankar 2014). Anthocyanins are mainly found in sweetpotato cultivars with purple leaves. According to a review, fifteen anthocyanin compounds, called acylated cyanidins and peonidins, were found in sweetpotato leaves (Islam, 2006). Acylated cyanidin contents were higher than those of acylated

peonidin, and acylated cyanidins had higher antioxidant and antimutagenicity capabilities. Studies have suggested that sweetpotato leaf anthocyanins contain anti-inflammatory and anti-cancer properties (Islam, 2006; Kurata et al., 2007).

Besides anthocyanins, there are other flavonoids present in SPL, including flavones (Zhao *et al.,* 2007; Ojong *et al.,* 2008). Zhao *et al.* (2007) identified flavones in SPL through the aluminum nitrate reagent method. Because the reagent might react with other polyphenols, it is important to question the accuracy of this flavone identification. Moreover, no further qualitative, or quantitative analysis such as liquid or gas chromatography was performed to confirm flavone presence (Zhao *et al.,* 2007).

Carotenoids and Chlorophylls

Although polyphenols are the major compounds in sweetpotato leaves, SPLs are known to contain the carotenoids lutein and β-carotene as well as chlorophyll which is responsible for the green pigment. In Li *et al* (2017), the leaves and stalks of fourteen sweetpotato cultivars were analyzed for lutein, β-carotene, and chlorophyll. The quantities of the compounds differed significantly between cultivars, with the leaves having higher concentrations of the three compounds than the stalks. In the leaves, lutein, β-carotene, and total chlorophyll contents ranged from 19.01 - 28.85, 35.21 - 52.01, and 440 - 712.2 mg /100 g DW, respectively. Lutein contents of the leaves were more than 10 times higher than of the stalks (1.88 – 3.77 mg/100 g DW), β-carotene of the stalks ranged from 0.48 to 2.57 mg / 100 g DW, and total chlorophyll contents of the leaves were 7.6-14.7 times higher than of the stalk (36.8 - 81.2 mg/100 g DW). The Healthymi cultivar possessed the highest level of lutein, β-carotene, and total chlorophyll in the leaves (Li *et al.,* 2017).

3. Health Effects of SPL

Sweetpotatoes have a long history, starting from their native home in the Americas, where they were historically used to make sweetpotato leaf decoction to treat mouth and throat tumors (Abidin, 2004). Although different parts of the *Ipomoea batatas* plant have separate medicinal purposes, the leaf is found to be a source of lutein, used to prevent, and treat age-related macular degeneration (Abidin, 2004). In Himalayan tribes and Malaysia, the sweetpotato plant juice and leafy tops have been utilized to control hyperglycemia in diabetics (Chhetri *et al.,* 2005*).* *Ipomoea batatas* leaf was documented to protect eyesight, to prevent atherosclerosis, mutagenesis, and carcinogenesis, and to accelerate metabolism. In support, leaves in the study were indeed found to effectively control hyperglycemia in diabetic rats (Zhao *et al.,* 2007).

3.1 Antioxidants

Ipomoea batatas leaves are known to contain significant antioxidant activity (Koncic *et al.,* 2012). Oxidative stress induces major damage to cells, and antioxidant activity can reduce the production of harmful, reactive oxidative species that potentiate the progression of diseases from diabetes to cancer. Various antioxidant assays were performed, including SPL scavenging effect on DPPH free radical, reducing power, β-carotene-linoleic acid assay, and superoxide dismutase-like activity, and SPL demonstrated strong antioxidant activity in the various assays (Koncic *et al.,* 2012). DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) is a colorimetric assay involving a chemical reaction where the sample antioxidant scavenges the free radical electron in the DPPH compound, reducing DPPH in a detectable color change reaction. Sweetpotato leaves had a higher DPPH radical scavenging activity than garland chrysanthemum, spinach, broccoli, cabbage, and lettuce (Kurata *et al.,* 2007). Sweetpotato leaves are a good source of antioxidant polyphenols, such as mono-caffeoylquinic acids (caffeic acid and chlorogenic acid), di-

caffeoylquinic acids, and tri-caffeoylquinic acids (Islam, 2006). A study specifically identified caffeic acid in SPL to have the most antioxidant activity, with the mono-caffeoylquinic acids second, di-caffeoylquinic acids third, and 3,4,5-tri-O-caffeoylquinic acid last (Sun *et al.* 2018).

Sun *et al.* (2018) investigated the total and individual phenolic compounds for their *in vitro* antioxidant activity and their ability to inhibit intracellular reactive oxygen species (ROS) (Sun *et al.,* 2018). ROS are metabolic byproducts involved in degenerative pathological processes in humans. ROS overproduction accelerates the progression of diseases like cancer and diabetes (Sun *et al.* 2018). Although SPL could likely decrease the levels of intracellular ROS, the inhibiting effect of the individual SPL phenolic acids on ROS did not accordingly correlate with their respective antioxidant activities, indicating no solid correlation between antioxidant activity and ROS-inhibiting effect.

Additionally, in Hue *et al* (2012), six sweetpotato leaves cultivars were analyzed for total flavonoids, total phenolics, reducing activity, and free radical scavenging ability. Four different assays, Folin-Ciocalteau, Vanillin-HCl, ferric reducing antioxidant power (FRAP), and DPPH radical scavenging were used accordingly to determine these four antioxidant properties (Hue *et al.,* 2012). The leaf variety with the highest antioxidant properties, "var Indon", was then proposed as a suitable source of natural antioxidant to substitute the usage of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) synthetic antioxidants. The usage of synthetic antioxidants has been found to increase the risk of cancer and liver damage in humans (Hue *et al.,* 2012). Of the six-leaf varieties, five cultivars had stronger free radical scavenging activity than the ascorbic acid standard, indicating that SPL is on par or is a better antioxidant than Vitamin C (Hue *et al.,* 2012). In support, sweetpotato leaf of the cultivar Yuzi No. 7

possessed significantly higher antioxidant activity than ascorbic acid, tea polyphenols, and grape seed polyphenols (Sun *et al.,* 2018).

Vitamin C is among the most popular and powerful of antioxidants, even taken as a controversial cold remedy for its antioxidant power (Douglas and Hemila 2005) Sweetpotato leaves demonstrated similar or higher antioxidant activity than Vitamin C, indicating that it is favorable to conserve the often-discarded greens. Studies have reinforced that SPLs have high levels of nutrients and strong antioxidant activity when compared to popular antioxidant-containing foods, demonstrating the market and nutritive potential for SPL products (Hue *et al.,* 2012).

3.2 Anti-Cancer Effects

In vitro studies have found that sweetpotato leaves possess anti-proliferative and anti-tumor qualities in the prostate, stomach, and colon cancers (Kurata *et al.,* 2007; Vishnu *et al.,* 2019). However, as far as it is known, the only published *in vivo* animal studies of sweetpotato leaves have been conducted in prostate cancer xenografts (Karna *et al.,* 2011; Gundala *et al.,* 2013).

Prostate cancer is common cancer and leading cancer in deaths in developed nations such as the United States (Haas *et al.,* 2008). However, the incidence is influenced by the intensity of diagnosis and the reliability of cancer registries. Although prostate cancer is leading cancer in the United States, the United States also has one of the top early detection programs and the highest incidence (Haas *et al.,* 2008). Prostate cancer prevalence ranks highest in Caucasian American and African American men, and the cancer is identified much earlier than expected, without affecting old age. Thus, prostate cancers undergo latency for up to 15 to 20 years. In this period, the cancer tissue is present as a benign tumor, but it has not proliferated and metastasized.

However, some prostate cancers can be much more aggressive and have a shorter, if any, latency period (Haas *et al.,* 2008). Publications of the anti-cancer properties of sweetpotato leaves have focused on the effects of SPL polyphenols against prostate cancer cell lines and in *in vivo* animal studies (Karna *et al.,* 2011; Gundala *et al.,* 2013). However, one gap of knowledge in this research papers is that they cannot factor in latency in animal models because of the short time frame of animal studies. Therefore, epidemiological studies could be better geared towards investigating latency.

Because sweetpotato leaves have high polyphenol content compared with several commercial vegetables including spinach, mustard greens, kale, green onions, and collard greens (Karna *et al.,* 2011), SPL polyphenols were investigated for anti-prostate cancer activities that these researched vegetables and leafy greens possess (Karna *et al.,* 2011). These measurements were estimated in terms of chlorogenic acid (ChA) equivalents, a common standard, with sweetpotato greens having polyphenolic concentrations 43% higher (in mg/L) than spinach. The richness of polyphenols contributes to antiproliferation against prostate cancer cells *in vitro* and *in vivo* (Karna *et al.,* 2011).

The mechanism of apoptosis of the prostate cancer cell line induced by SPL was investigated. The sweetpotato green extract (SPGE) caused prostate cancer inhibition by G_1 phase arrest followed by a mitochondrially mediated caspase-dependent intrinsic apoptosis in PC-3 prostate cancer cells *in vitro* (Karna *et al.,* 2011). The *in vivo* studies showed that the extracts inhibit tumor growth of subcutaneously implanted PC-3 human tumor xenografts in nude mice without toxic side effects (Karna *et al.,* 2011).

In a supporting study, the researchers proved the growth-inhibitory and apoptosis-inducing capabilities of polyphenol-rich sweetpotato greens extract (SPGE) in *in vitro* cell cultures

and *in vivo* prostate cancer xenograft models (Gundala *et al.,* 2013). “Xenograft” refers to the transplantation of tissue from one species to a dissimilar species (or a species of a different genus or family). Xenografts are common in animal studies investigating cancer proliferation. The neoplasm (tumor) from a human can be transferred and grown on mice and rats. Then, the animal is treated, and the neoplasm is measured for change in size and proliferation. It is not an exact model, as a human neoplasm in a rat or mice will not react the same or have the same mechanism as a neoplasm in a human body.

The treatment in Gundala *et al.* (2013) was an active polyphenol-enriched fraction called F_5, which was one-hundred times stronger than the initial sweetpotato green (SPG) extraction measured by IC_{50} (concentration at which half the cells were inhibited) of human prostate cancer cells (Gundala *et al.,* 2013). By HPLC-ultraviolet and mass spectrometric analyses, the authors found quinic acid (QA), caffeic acid, chlorogenic acid, isochlorogenic acid, 4,5-di-CQA, 3,5-di-CQA, and 3,4-di-CQA. The F_5 fraction particularly had higher amounts of QA and chlorogenic acids. In the animal study, 400 mg of F_5 per kilogram of body weight of nude mice was administered daily by the oral route. The tumor volumes were measured, and non-invasive real-time bioluminescence imaging was conducted. The dosage inhibited growth and progression of prostate tumor xenografts by about 75% in the nude mice (Gundala *et al.,* 2013). Nude mice are commonly used in xenograft research because they can receive different types of tissue and tumor grafts without an immune response. This is because they come from a strain with a genetic mutation that results in a damaged or missing thymus gland, which thus inhibits their immune systems.

Kurata *et al.* (2007) examined growth suppression of stomach, leukemia, and colon cancer cell lines by SPL polyphenols (Kurata *et al.,* 2007). Stomach cancer is cancer that typically starts

in the mucus-secreting cells of the stomach lining (adenocarcinoma). Rates of stomach adenocarcinoma have been dropping worldwide in the last decades. However, rates of cancer between the top part of the stomach (cardia) and the esophagus have increased (Niederhuber *et al.,* 2013). This stomach area is called the gastroesophageal junction. Leukemia, also called blood cancer, is a cancer of blood-forming tissues, such as the blood marrow. Because the blood marrow is a primary organ of the immune system, leukemia hinders the body's ability to fight infection.

In Kurata *et al.,* (2007), caffeic acid and the di-and tri-caffeoylquinic phenolic acids isolated from SPLs collectively dose-dependently depressed cancer cell proliferation in all three cancer cell lines: stomach, colon, and leukemia. 3,4,5-tri-O-caffeolylquinic acid was most effective in depressing the growth of all three cancer cell lines collectively. According to Sun *et al.* (2018), 3,4,5-tri-*O*-caffeoylquinic had the lowest antioxidant activity in comparison to the other caffeoylquinic acid derivatives, but in the study (Kurata *et al.,* 2007), 3,4,5-tri-*O*-caffeoylquinic acid had the highest combined anti-cancer effect (in all cell lines). It should be investigated if antioxidant capacities of SPL compounds are correlated to their anti-cancer effects. Caffeic acid alone had a higher depressive effect against the promyelocytic leukemia cell line (HL-60). It was found that the acid-induced apoptosis through observed nuclear granulation and increase of caspase-3 activity and *c-Jun* expression. This was confirmed by DNA fragmentation (Kurata *et al.,* 2007). Caspase-3 belongs to a family of apoptotic enzymes, and *c-Jun* is a gene expressed during programmed cell death.

The stomach cancer cell line used in the study (Kurata *et al.,* 2007), Kato III, is a human gastric carcinoma cell line and is dissimilar from stomach cancer cells currently increasing in cancer patients. Similarly, the leukemia cell line (HL60) used was isolated from a patient with

acute promyelocytic leukemia. Cancer cell lines are derived and established *in vitro* from patients, and represent special, rare cases when the cancer cell lines have been successfully immortalized. Therefore, cancer cell line research is lacking and needs to be progressed with *in vivo* animal studies, human, and epidemiological studies. This study (Kurata *et al.,* 2007) was the first to provide data that SPL CQA derivatives dose-dependently depressed growth of stomach and leukemia cancer cell lines. Few other studies (Vishnu *et al.,* 2019, Taira *et al.,* 2014) have investigated SPL effects against colon cancer cell lines.

Anthocyanins in purple sweetpotato leaf were investigated as having anti-cancer properties (Vishnu *et al.,* 2019). Anthocyanins have been indicated to possess antioxidant activity, as well as induce apoptosis of and suppress tumor cells (Vishnu *et al.,* 2019). Cyanidin is the primary anthocyanin in certain sweetpotato leaves varieties in Japan. In the study, a Japanese variety of SPL called Bhu Krishna exhibited potential antiproliferative against MCF-7 (breast cancer), HCT-116 (colon cancer), and HeLa (cervical cancer) cancer cell lines, with anthocyanin structure as the major suppressive agent. The leaf anthocyanins demonstrated higher suppression against colon and cervical cancer cells, and lower against breast cancer (Vishnu *et al.,* 2019).

Not many other studies have studied the anti-cancer properties of the *Ipomoea Batatas* leaf (Taira *et al.,* 2014; Kurata *et al.,* 2007). Most focus on the anti-cancer qualities of the sweetpotato root. One study identified pectin, a soluble fiber, in the sweetpotato root peel as capable of inducing apoptosis-like cell death in human colon cancer (HT-29) cell line (Zaidel *et al.,* 2017). Pectin, specifically pectin oligosaccharides (POS), have anti-colon cancer properties among several plants. This study showed that POS alleviate colon cancer by controlling oxidative stress and inflammation-stimulated signaling pathways (Tan *et al.,* 2018).

3.3 Anti-Mutagenicity

Cancer researchers generally accept the “two-step carcinogenesis” hypothesis that a cancer cell proliferates from a healthy one by steps beginning with mutation and promotion. Later, if cancer has metastasized, chances of remission and/or survival decrease, so prevention of mutation is key (Kurata *et al.,* 2007).

Claiming anti-mutagenic includes, but is not limited to solely anti-carcinogenic. According to Bhattachar (2011), mutagens are also involved in the origin and pathogenesis of several chronic degenerative diseases including hepatic disorders, neurodegenerative disorders, cardiovascular disorders, diabetes, arthritis, chronic inflammation, and aging (Bhattachar, 2011). Naturally occurring antimutagenic compounds present in plants have protective effects against mutagens. These include flavonoids, phenolics, carotenoids, tannins, and saponins. Furthermore, since the early 2000s, screening methods have allowed detection of various inhibitors that act against mutagens and carcinogens in our daily diet, which play an important part in preventing mutations and cancer (Yoshimoto *et al.,* 2002).

A study examined the antimutagenicity of caffeoylquinic acid (CQA) derivatives, classified under phenolic acids (Yoshimoto *et al.,* 2002). These included caffeic acid, chlorogenic acid, 3,4-diCQA, (3,4-dicaffeyolqunic acid), 3,5-diCQA, 4,5-diCQA, and 3,4,5-triCQA from sweetpotato leaves from Japan. These phenolic acids effectively inhibited the mutagenicity induced in *Salmonella uityphimurium* (TA98 bacterial strand) by the carcinogen Trp-P-1. This mutation of the bacterial genome occurs in the overcooking of meat or fish. 3,4,5-triCQA was the most inhibitory antimutagenic followed by a three-way tie between the di-caffeoylquinic acids, and with chlorogenic acid last. The three di-caffeoylquinic acid derivatives and 3,4,5-tri-CQA were about 1.5 to 2.0 times higher than chlorogenic acid in % inhibition. Because of the correlation

between structural complexity and antimutagenicity, the study compared structures and activities, finding that the number of caffeoyl groups bound to quinic acid played a role in the degree of antimutagenicity (Yoshimoto *et al.,* 2002). Via apoptosis, 3,4,5-triCQA was the most successful in suppressing three human cancer cell lines in Kurata *et al.* (2007), followed by the di-CQAs.

Anthocyanins, mainly cyanidins and peonidins, found in purple sweetpotato leaf varieties were also found by two studies to have antimutagenic properties (Yoshimoto *et al.,* 2002; Islam, 2006). Cyanidin had stronger antimutagenic activity than peonidin, the other anthocyanin present, due to that cyanidin contains one more hydroxyl group than peonidin (Yoshimoto *et al.,* 2002). The study found that anthocyanins prevent mutagen formation by reacting with enzymatically activated carcinogens called heterocyclic amines (Yoshimoto *et al.,* 2002).

3.4 Anti-Diabetic Effects

Diabetes mellitus is a major health problem, caused by the improper maintenance of glucose and insulin. Diabetes mellitus has been steadily increasing in the U.S. over the last twenty years and 8% of the population, roughly 25.8 million people, had been diagnosed. A healthy, vegetable-rich diet is important to maintain body weight and a crucial risk factor for diabetes (Poquette *et al.,* 2014).

The effects of flavone, a class of flavonoid, from *Ipomoea Batatas* leaf on body weight, blood glucose, serum lipid profiles, serum insulin, and free radicals on non-insulin-dependent diabetic rats were studied (Zhao *et al.,* 2007). Flavone treatment for two weeks significantly decreased the weight and amounts of plasma triglycerides and plasma cholesterol in the type-2 diabetic rats. Additionally, there was a slight decrease in fasting plasma insulin, blood glucose, LDL cholesterol, and, malondialdehyde concentrations, while insulin sensitivity was increased.

Although just a short-term animal study, these results showed that SPL can control blood glucose and modulate lipid metabolism in non-insulin-dependent diabetic rats (Zhao *et al.,* 2007). Anti-diabetic properties of SPL were evaluated in streptozocin-induced diabetic rats (Rafiu and Cd, 2018). In SPL-treated rats, the treated group showed a significant decrease in glucose, total cholesterol, triglyceride, LDL, and lipid peroxidase activity (Rafiu and Cd, 2018).

4. SPL Toxicity

4.1 Anti-Fertility

Through research, it has been established that SPLs have a variety of nutrients and antioxidant activity, but the health effects of SPL in connection to specific diseases is a larger area that demands further investigation. An animal study examined the effect of SPL extract on sperm toxicity and weight of tests and epididymis (duct behind testes) in male albino rat models (Uno *et al.,* 2017). Twenty-four male rats were split into four groups, A, B, C, D (6 rats each) using a completely randomized design over 65 days. Group A was the control, and the rats were fed only water and pellet feed. Group B received 200 mg / kg, Group C received 400 mg / kg, and Group D received 600 mg / kg body weight of *I. batatas* leaf aqueous extract. The results showed a significant decrease in the epididymis weight, sperm motility, sperm viability, and sperm count while sperm head abnormalities significantly increased in the treat rats. However, no significant difference in the semen pH and weight of the testes was observed. The data suggest that aqueous SPL extract has a toxic effect on the sperm of male albino rats in a dose-dependent manner (Uno *et al.,* 2017).

The findings of Uno *et al* (2017) agree with a former study investigating the effect of SPL extract on the thyroid gonadal axis of male rats (Udoh *et al.,* 2010). In this study, an aqueous

extract of *I. batatas* leaf was also prepared which was administered orally in three doses (100, 200, and 300 mg / kg body weight / day) for three weeks. At the end of the three weeks, the rats were sacrificed, and their testes and thyroid glands were isolated. After tissue histopathology analysis, hypertrophy, and hyperplasia (kinds of enlargement) of the follicles was revealed, in addition to reduction of colloid in the lumen of the follicles. Evaluation of testes morphology showed oligospermia (low sperm count), athenospermia (reduced sperm motility), and abnormal sperm morphology suggesting that the SPL extract had anti-fertility properties (Udoh *et al.,* 2010). However, rat models are often not transferable to human models, and humans could display less or negligible SPL spermatotoxic effects.

4.2 Oxalate Toxicity

Moreover, a review brings attention to possibly toxic oxalate concentrations in sweetpotato leaves (Islam, 2006). High intake of oxalate can induce acute poisoning, resulting in hypocalcemia, or poisoning where calcium oxalate forms as crystals in the kidneys, causing renal damage (Islam, 2006). Oxalate binding to calcium reduces calcium bioavailability, as calcium oxalate is poorly utilized by humans. In the study, the sweetpotato leaves of the 'Suioh' cultivar contained 280 mg oxalate/100 g fresh weight (Islam, 2006).

In comparison, spinach, also a leafy green vegetable, contained 658 mg oxalate / 100 g fresh weight serving (Han *et al.,* 2015). In a large cohort (~ 46,000 men, ~ 102,000 younger women, ~ 93,000) study over 44 years to investigate the effect of dietary oxalate intake on oxalate stone risk, the average oxalate consumption was 214 mg / day for men, 185 mg / day for older women, and 183 mg / day for younger women. Spinach accounted for more than 40% of total oxalate intake. However, the group in the top quintile of dietary oxalate intake only had a small risk

increase for the incidence of kidney stones in models fixed for age, body mass index, use of thiazide diuretics, and other dietary compounds (Taylor and Curhar 2007).

References

Abidin, P. E. (2004). *Sweetpotato breeding for northeastern Uganda: Farmer varieties, farmer-participatory selection, and stability of performance*.

Arora, Sharma, & Tollefsbol. (2019). Combinatorial Epigenetics Impact of Polyphenols and Phytochemicals in Cancer Prevention and Therapy. *International Journal of Molecular Sciences*, *20*(18), 4567.

Bhattachar, S. (2011). Natural Antimutagens: A Review. *Research Journal of Medicinal Plant*, *5*(2), 116–126.

Boutrif, E. (1991). "Recent Developments in Protein Quality Evaluation" Food, Nutrition and Agriculture, Issue 2/3. *Food Quality and Consumer Protection Group, Food Policy and Nutrition Division, FAO, Rome:*

Bunel, V., Ouedraogo, M., Nguyen, A., Stévigny, C., & Duez, P. (2014). Methods Applied to the In Vitro Primary Toxicology Testing of Natural Products: State of the Art, Strengths, and Limits. *Planta Medica*, *80*(14), 1210–1226.

Chhetri, D. R., Parajuli, P., & Subba, G. C. (2005). Antidiabetic plants used by Sikkim and Darjeeling Himalayan tribes, India. *Journal of Ethnopharmacology*, *99*(2), 199–202.

Douglas, R. M., & Hemilä, H. (2005). Vitamin C for Preventing and Treating the Common Cold. *PLoS Medicine*, *2*(6), e168.

Favoriti, P., Carbone, G., Greco, M., Pirozzi, F., Pirozzi, R. E. M., & Corcione, F. (2016). Worldwide burden of colorectal cancer: A review. *Updates in Surgery*, *68*(1), 7–11.

Feldman, M., Friedman, L. S., & Brandt, L. J. (Eds.). (2020). *Sleisenger and fordtran's gastrointestinal and liver disease: Pathophysiology, diagnosis, management* (11th ed.). Philadelphia: Elsevier.

Food and Agriculture Organization of the United Nations (FAO). (2020, June 15). FAOSTAT Crops. Retrieved from http://www.fao.org/faostat/en/#data/QC

Galanakis, C. M. (2017). Introduction. *Nutraceutical and Functional Food Components* (pp. 1–14). Elsevier. Retrieved May 15, 2020, from https://linkinghub.elsevier.com/retrieve/pii/B9780128052570000016

Gandomani, H. S., Yousefi, S. M., Aghajani, M., Mohammadian-Hafshejani, A., Tarazoj, A. A., Pouyesh, V., & Salehiniya, H. (2017). Colorectal cancer in the world: Incidence, mortality and risk factors. *Biomedical Research and Therapy*, *4*(10), 1656.

Gundala, S. R., Yang, C., Lakshminarayana, N., Asif, G., Gupta, M. V., Shamsi, S., & Aneja, R. (2013). Polar biophenolics in sweet potato greens extract synergize to inhibit prostate cancer cell proliferation and in vivo tumor growth. *Carcinogenesis*, *34*(9), 2039–2049.

Haas, G. P., Delongchamps, N., Brawley, O. W., Wang, C. Y., & de la Roza, G. (2008). The worldwide epidemiology of prostate cancer: Perspectives from autopsy studies. *The Canadian Journal of Urology*, *15*(1), 3866–3871.

Haggar, F., & Boushey, R. (2009). Colorectal Cancer Epidemiology: Incidence, Mortality, Survival, and Risk Factors. *Clinics in Colon and Rectal Surgery*, *22*(04), 191–197.

Huang, X., Tu, Z., Xiao, H., Li, Z., Zhang, Q., Wang, H., Hu, Y., et al. (2013). Dynamic high pressure microfluidization-assisted extraction and antioxidant activities of sweet potato (Ipomoea batatas L.) leaves flavonoid. *Food and Bioproducts Processing*, *91*(1), 1–6.

Huang, Z., Wang, B., Eaves, D. H., Shikany, J. M., & Pace, R. D. (2007). Phenolic compound profile of selected vegetables frequently consumed by African Americans in the southeast United States. *Food Chemistry*, *103*(4), 1395–1402.

Hue et al. (2012). Antioxidant activity, phenolic and flavonoid contents in the leaves of different varieties of sweet potato (Ipomoea batatas). *Australian Journal of Crop Science*, *6*(3), 375–380.

Ishida, H. (2000). Nutritive evaluation on chemical components of leaves, stalks and stems of sweet potatoes (Ipomoea batatas poir). *Food Chemistry*, *68*(3), 359–367.

Islam, Md. S., Yoshimoto, M., Yahara, S., Okuno, S., Ishiguro, K., & Yamakawa, O. (2002). Identification and Characterization of Foliar Polyphenolic Composition in Sweetpotato (*Ipomoea batatas* L.) Genotypes. *Journal of Agricultural and Food Chemistry*, *50*(13), 3718–3722.

Islam, S. (2006). Sweetpotato (Ipomoea batatas L.) Leaf: Its Potential Effect on Human Health and Nutrition. *Journal of Food Science*, *71*(2), R13–R121.

Johnson, M., & Pace, R. D. (2010). Sweet potato leaves: Properties and synergistic interactions that promote health and prevent disease: Nutrition Reviews©, Vol. 68, No. 10. *Nutrition Reviews*, *68*(10), 604–615.

Karna, P., Gundala, S. R., Gupta, M. V., Shamsi, S. A., Pace, R. D., Yates, C., Narayan, S., et al. (2011). Polyphenol-rich sweet potato greens extract inhibits proliferation and induces apoptosis in prostate cancer cells in vitro and in vivo. *Carcinogenesis*, *32*(12), 1872–1880.

Koncic, M. Z., Petlevski, R., & Kalođera, Z. (2013). Antioxidant Activity of *Ipomoea batatas* L. Lam. Leaf Grown in Continental Croatia and Its Effect on Glutathione Level in Glucose-Induced Oxidative Stress. *International Journal of Food Properties*, *16*(5), 964–973.

Kurata, R., Adachi, M., Yamakawa, O., & Yoshimoto, M. (2007). Growth Suppression of Human Cancer Cells by Polyphenolics from Sweetpotato (*Ipomoea batatas* L.) Leaves. *Journal of Agricultural and Food Chemistry*, *55*(1), 185–190.

Li, M., Jang, G. Y., Lee, S. H., Kim, M. Y., Hwang, S. G., Sin, H. M., Kim, H. S., et al. (2017). Comparison of functional components in various sweet potato leaves and stalks. *Food Science and Biotechnology*, *26*(1), 97–103.

Ligor, M., Trziszka, T., & Buszewski, B. (2013). Study of Antioxidant Activity of Biologically Active Compounds Isolated from Green Vegetables by Coupled Analytical Techniques. *Food Analytical Methods*, *6*(2), 630–636.

Miean, K. H., & Mohamed, S. (2001). Flavonoid (Myricetin, Quercetin, Kaempferol, Luteolin, and Apigenin) Content of Edible Tropical Plants. *Journal of Agricultural and Food Chemistry*, *49*(6), 3106–3112.

Mohanraj, R., & Sivasankar, S. (2014). Sweet Potato (*Ipomoea batatas* [L.] Lam) - A Valuable Medicinal Food: A Review. *Journal of Medicinal Food*, *17*(7), 733–741.

Morgan, D. O. (2007). *The cell cycle: Principles of control*. Primers in biology. London : Sunderland, MA: Published by New Science Press in association with Oxford University Press ; Distributed inside North America by Sinauer Associates, Publishers.

Niederhuber, J. E., Armitage, J. O., Doroshow, J. H., Kastan, M. B., Tepper, J. E., & Abeloff, M. D. (2014). *Abeloff's clinical oncology* (Fifth edition.). Philadelphia, Pennsylvania: Elsevier.

Ojong, P. B., Njiti, V., Guo, Z., Gao, M., Besong, S., & Barnes, S. L. (2008). Variation of Flavonoid Content Among Sweetpotato Accessions. *Journal of the American Society for Horticultural Science*, *133*(6), 819–824.

Poquette, N. M., Gu, X., & Lee, S.-O. (2014). Grain sorghum muffin reduces glucose and insulin responses in men. *Food Funct.*, *5*(5), 894–899.

Rafiu, A., & Cd, L. (2018). Blood Electrolytes, and Enzymatic Antioxidants of Streptozotocin-Induced Diabetic Rats. *International Journal of Diabetes and Metabolism*, *4*, 21–026.

Rawla, P., Sunkara, T., & Barsouk, A. (2019). Epidemiology of colorectal cancer: Incidence, mortality, survival, and risk factors. *Gastroenterology Review*, *14*(2), 89–103.

Sun, H., Mu, B., Song, Z., Ma, Z., & Mu, T. (2018). The *In Vitro* Antioxidant Activity and Inhibition of Intracellular Reactive Oxygen Species of Sweet Potato Leaf Polyphenols. *Oxidative Medicine and Cellular Longevity*, *2018*, 1–11.

Sun, H., Mu, T., Xi, L., Zhang, M., & Chen, J. (2014). Sweet potato (Ipomoea batatas L.) leaves as nutritional and functional foods. *Food Chemistry*, *156*, 380–389.

Taira, J., Uehara, M., Tsuchida, E., & Ohmine, W. (2014). Inhibition of the β-Catenin/Tcf Signaling by Caffeoylquinic Acids in Sweet Potato Leaf through down Regulation of the Tcf-4 Transcription. *Journal of Agricultural and Food Chemistry*, *62*(1), 167–172.

Tan, H., Chen, W., Liu, Q., Yang, G., & Li, K. (2018). Pectin Oligosaccharides Ameliorate Colon Cancer by Regulating Oxidative Stress- and Inflammation-Activated Signaling Pathways. *Frontiers in Immunology*, *9*, 1504.

Taylor, E. N., & Curhan, G. C. (2007). Oxalate Intake and the Risk for Nephrolithiasis. *Journal of the American Society of Nephrology*, *18*(7), 2198–2204.

Udoh et al. (2010). Effect of Ipomoea batatas leaf extract on the thyroid-gonadal axis of male Wistar rats. *Journal of Physiology and Pathophysiology*, *1*(2), 18–21.

United Nations. (2019). Population. *Global Issues*. Retrieved from https://www.un.org/en/sections/issues-depth/population/

United States Department of Agriculture (USDA). (2019, May 4). Statistics by Subject. *National Agricultural Statistics Service*. Retrieved from https://www.nass.usda.gov/Statistics_by_Subject/result.php?3BADBCBE-0F43-3DAD-AE40-449AB25BCFF1§or=CROPS&group=VEGETABLES&comm=SWEET%20POTATOES

Uno, U., Okolo, C., Ogbe, H., Ekaluo, U., & Benjamin, M. (2017). Evaluation of Spermatotoxic Effect of Sweet Potato (Ipomoea batatas) Leaf Extract on Male Albino Rats. *Asian Journal of Biotechnology and Bioresource Technology*, *1*(3), 1–5.

U.S. Department of Agriculture (USDA). (2020, April 1). Sweet Potato, NFS. *FoodData Central Search Results*. Retrieved from https://ndb.nal.usda.gov/fdc-app.html#/food-details/787640/nutrients

Verhoeckx, K., Cotter, P., López-Expósito, I., Kleiveland, C., Lea, T., Mackie, A., Requena, T., et al. (Eds.). (2015). *The Impact of Food Bioactives on Health*. Cham: Springer International Publishing. Retrieved October 17, 2020, from http://link.springer.com/10.1007/978-3-319-16104-4

Vishnu, V. R., Renjith, R. S., Mukherjee, A., Anil, S. R., Sreekumar, J., & Jyothi, A. N. (2019). Comparative Study on the Chemical Structure and *In Vitro* Antiproliferative Activity of Anthocyanins in Purple Root Tubers and Leaves of Sweet Potato (*Ipomoea batatas*). *Journal of Agricultural and Food Chemistry*, *67*(9), 2467–2475.

Yoshimoto, M., Yahara, S., Okuno, S., Islam, Md. S., Ishiguro, K., & Yamakawa, O. (2002). Antimutagenicity of Mono-, Di-, and Tricaffeoylquinic Acid Derivatives Isolated from Sweetpotato (*Ipomoea batatas* L.) Leaf. *Bioscience, Biotechnology, and Biochemistry*, *66*(11), 2336–2341.

Zaidel, D. N., Hamidon, N. H., & Mat Zahir, N. (2017). Extraction and characterization of pectin from sweet potato (*Ipomoea batatas*) peels using alkaline extraction method. *Acta Horticulturae*, (1152), 211–218.

Zhang, Y., Chen, X., Gueydan, C., & Han, J. (2018). Plasma membrane changes during programmed cell deaths. *Cell Research*, *28*(1), 9–21.

Zhao, R., Li, Q., Long, L., Li, J., Yang, R., & Gao, D. (2007). Antidiabetic activity of flavone from Ipomoea Batatas leaf in non-insulin dependent diabetic rats. *International Journal of Food Science & Technology*, *42*(1), 80–85.

CHAPTER 2: A Study on The Antioxidant Properties and Anti-Colon Cancer Effect of Polyphenols from Sweetpotato Leaves (SPL) of Different Varieties

1. Abstract

Sweetpotato leaves (SPL) are a good source of dietary fiber, vitamins, minerals, and phytochemicals. Although several studies have characterized the bioactive compounds and nutritional content of SPLs, few have shown the health-promoting properties of SPL polyphenols. The objectives of the present study were to 1) determine the impact of year and variety on SPL polyphenol composition and antioxidant capacity, and to 2) investigate the anti-colon cancer effects of SPLs by Caco-2 colon cancer cell viability and apoptosis assays. In 2018 and 2019 SPLs, the total polyphenol content of the extracts was determined by Folin-Ciocalteu method, antioxidant capacity of the extracts was determined by DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method, and the top five 2019 SPL varieties (SPL1, SPL3, SPL9, SPL11, SPL28) were then selected for quantification of the major bioactive compounds. Viability and apoptosis effects of the five 2019 SPL varieties were evaluated using Cell Proliferation Assay and Cell Death Detection ELISA kit, respectively. Results showed that the 2019 SPL possessed higher average polyphenol content and antioxidant capacity than the 2018 SPL. 2019 SPL1 and SPL3 varieties had the highest total polyphenol and antioxidant capacity ($p < 0.05$). All five 2019 SPL varieties dose-dependently inhibited Caco-2 viability and increased Caco-2 apoptosis as compared to the negative control ($p < 0.05$). These findings indicate that the inhibitory effects of SPL on colon cancer cells may provide a potential application as a nutraceutical or functional food.

2. Introduction

Sweetpotato leaves (SPL), a by-product of the sweetpotato plant, contain substantial amounts of vitamins, minerals, and bioactive compounds that may possess health-promoting properties that are not well-investigated (Hue *et al.,* 2012). Studies have shown that SPLs have an antioxidant capacity, due mostly to their polyphenol content. The major polyphenols in SPLs are phenolic acids, called the caffeoylquinic acid (CQA) derivatives (Islam et al., 2002). However, different SPL cultivars vary in polyphenol content and antioxidant capacity, as well as individual polyphenol composition (Krochmal-Marczak *et al.,* 2020).

Colorectal cancer (CRC) is a threat to health, being the third most common cancerous malignancy and the fourth leading cause of cancer-related deaths worldwide, totaling 700,000 annual deaths worldwide (Favoriti *et al.* 2016). SPLs have been shown to have *in vitro* anti-colon cancer effects (Taira *et al.,* 2013; Kurata *et al.,* 2007; Vishnu *et al.,* 2019). Kurata *et al.* (2007) demonstrated that the CQA derivatives in SPLs contained anti-cancer activities against the DLD-1 colon cancer cell line by inhibiting cancer cell viability. However, these effects have not been shown in a Caco-2 colon cancer cell model. Caco-2 cells are a good model of cancer of the intestines as they express the enzymes and transporters of primary epithelial cells (Verhoeckx *et al.,* 2015). Apoptosis is the most common form of eukaryotic cell death (Bunel *et al.,* 2013), and studies have shown that extracted phenolic acids decrease cancer cell viability and increase apoptosis (Yi *et al.,* 2005). However, the effect of SPL polyphenols has not been investigated on Caco-2 cells, and more studies are needed with different cell models to confirm the effect and mode of action of SPLs against colon cancer.

Thus, the **hypothesis** was that the SPL varieties with the highest amounts of polyphenols would decrease Caco-2 cancer cell viability and increase apoptosis. The **objectives** were to

measure the SPL of year and variety by their polyphenol content and antioxidant capacity, and five SPLs varieties with the highest polyphenol contents were analyzed by HPLC to identify the major compounds. Last, the effects of five SPLs varieties on cell viability and apoptosis were evaluated.

3. Materials and Methods

3.1. Preparation of SPL Extracts

The sweetpotato accessions used in this study were obtained from the USDA national germplasm center (Plant Genetic Resources Conservation Unit). Sweetpotato roots were planted 2 inches deep and about 2 inches apart (density of 5 cm x 5 cm) in a greenhouse and field conditions in late February (greenhouse) to April (field) in the University of Arkansas at Pine Bluff's (UAPB) Agricultural Research Farm, Pine Bluff, Arkansas during 2018 and 2019. Also, the slips from sweetpotato accessions were planted in sterilized Pro-mix soil in 12 cm vinyl pots with proper tags at the greenhouse. After two months, tips were harvested every 15 days. Chemical fertilizer (N: P: K = 8: 8: 8) was used at a rate of 500 pounds/acre, and compost was used at a rate of 8000 pounds/acre in volume. After each harvest, 150 pounds/acre of ammonium sulfate was applied as additional fertilizer. Soap water was applied once a week to control Aphids. The greenhouse harvested leaves were weighed, washed with running tap water, packed in polyethylene bags, labeled, and frozen at -81 °C. Samples were freeze-dried using the MillRock Technology Freeze Dryer (MD3053, USA) and ground to powder using a Hamilton Beach Coffee Grinder (80333, China), weighted and stored in polyethylene bags. To obtain the polyphenolic extract, 0.2 grams of each SPL variety was shaken using a Thermo Scientific Max Q 2000 orbital shaker (Thermo Fisher Scientific, Waltham, MA) with 5 mL of 70% ethanol for 2

hours. After shaking, the extracts were vacuum filtered through Whatman 42 filter paper, and the supernatants were adjusted to 10 mL with 100% ethanol.

3.2 SPL Total Polyphenols and Antioxidant Capacity

Total polyphenol content was determined through a modified Folin-Ciocalteu assay method (Slinkard and Singleton, 1977). A gallic acid standard curve was obtained in concentrations of 100, 50, 25, 12.5, 6.25, and 3.125 ppm in deionized (DI) water. SPL samples were diluted 15 times in DI water. 60 µL of sample, standard or DI water blank, 300 µL of 0.2 N Folin-Ciocalteu reagent, and 240 µL of 0.7 M sodium carbonate were added to each well of a 48-well microplate. After 2 hours of incubation at room temperature in a dark room, absorbances were read at 760 nm using a Synergy™ HT Multi-detection microplate reader (Bio-Tek Instruments, Inc., Winooski, Vermont). Polyphenol content was determined by dividing the absorbance by the linear regression of the gallic acid standard curve. All samples and standards were measured in at least triplicate.

Antioxidant capacity was determined by a modified DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) radical scavenging method (Akkari *et al.,* 2016). A standard curve of Trolox diluted in methanol of 500, 150, 50, 25, and 12.5 µmol was prepared. SPL samples were diluted 15 times in methanol. 30 µL of diluted SPL sample in methanol, standard, or methanol blank, and 420 µL of 0.1 mM DPPH solution in methanol were added to a 48-well microplate. After 30 minutes of incubation at room temperature in a dark room, absorbance was measured at 517 nm. Radical-scavenging activity was calculated using the equation: Scavenging effect = $[(A_0 - A_1)/A_0] \times 100$, where A_0 was the absorbance of the control and A_1 is the absorbance in the presence of the sample. Total antioxidant capacity was then determined by fitting the scavenging effect into the

linear regression line of the Trolox standard curve. All samples and standards were measured in at least triplicate.

3.3 Analysis of Major Bioactive Compounds in SPL

Standards

For caffeoylquinic acid analysis, a standard solution mixture containing chlorogenic acid, ≥ 98% purity (Quality Phytochemicals LLC, New Jersey, USA), 3,4-dicaffeoylquinic acid, ≥ 98% purity and 3,5-dicaffeoylqunic acid, ≥ 98% purity (Cayman Chemicals, Ann Arbor, MI) in 80% methanol was prepared. A 0.45 μm PTFE (polytetrafluoroethylene) syringe filter was used to filter the solution. A standard curve was obtained with concentrations of 100, 50, 25, 12.5, 6.25, 3.13, and 1.56 ppm. For carotenoid and chlorophyll analysis, chlorophyll A and chlorophyll B (Sigma-Aldrich, St. Louis, MO) and lutein (Cayman Chemical, Ann Arbor, MI) were solubilized in dichloromethane and diluted with 100% methanol. A 0.45 μm PTFE syringe filter was used to filter the solution. Standard curves were obtained with concentrations of 12.5, 6.3, 3.1, 1.6, 0.8, and 0.4 ppm for chlorophyll A and B, and of 20.8, 10.4, 5.2, 2.6, 1.3, and 0.7 ppm for lutein.

SPL Preparation and HPLC Analysis

Because samples in 2019 had higher average polyphenol content and antioxidant capacity than in 2018, 2019 five significantly highest samples in both polyphenol content and antioxidant capacity were selected for HPLC analysis. SPL1, SPL3, SPL9, SPL11, and SPL28 were extracted to obtain polyphenols under the same method as described before using 70% ethanol and shaking. After centrifuging the extract at 5000 rpm for 5 minutes, 1 mL (for polyphenol analysis) and 2 mL (for chlorophyll and carotenoid analysis) were evaporated under nitrogen gas (N-EVAP™ 111 Nitrogen Evaporator, Organomation Associates, Inc, Berlin, MA). After

evaporation, the sweetpotato leaf solids were re-dissolved in 80% methanol and diluted to a concentration of 0.5 mg/mL (polyphenol) or 2 mg/mL (chlorophyll and carotenoids). The samples were filtered through a 0.45 µm PTFE syringe filter.

Samples, 50 µL (polyphenols) and 100 µL (chlorophyll and carotenoids), were analyzed using a Shimadzu™ Prominence UFLC (Ultra-Fast Liquid Chromatography) (Shimadzu Corp., Kyoto, Japan) equipped with a model LC-20AB pump, a model SIL-10AF autosampler, and a model SPD-M20A photodiode array detector. The separation was carried out using either a YMC-Pack ODS-AM C18 column (250 mm x 10 mm, 5 µm) for polyphenols or a YMC C30 column (250 mm x 4.6 mm, 5 µm) for carotenoids and chlorophyll (YMC CO., LTD, Kyoto, Japan). For polyphenols, the mobile phase was a linear gradient of 0.2% formic acid in DI water (A) and methanol (B) from 5% to 75% B for 65 min at 1 mL/min. For carotenoids and chlorophyll, the mobile phase was a gradient of 100% methanol (A) and 100% dichloromethane (B) from 5 to 10% B over 10 minutes, increased to 20% B at 15 minutes, 40% B at 30 minutes, and to 60% B at 53 minutes for 65 min at 1 mL/min. Detection wavelengths were set at 326 nm for polyphenols and 450 nm for chlorophyll and carotenoids.

3.4 Cell Culture

The human colon adenocarcinoma cell line Caco-2 (ATCC® HTB-37™) was purchased from American Type Culture Collection (ATCC, Manassas, VA, USA) at passage number 19. Caco-2 were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% Non-Essential Amino Acids (NEAA), and 1% Antibiotic-Antimycotic. The cells were incubated in a 5% CO_2, 37 °C humidified incubator (VWR® Water Jacketed CO_2 incubator, VWR International, PA), and passaged every 3 days. All media components and reagents were obtained from Thermo Fisher Scientific (Waltham, MA, USA).

3.5 Cell Viability Assay

Cell viability was measured using the CellTiter 96® AQueous One Solution Cell Proliferation Assay (Promega Corporation, Madison, WI, USA). The reagent contains a tetrazolium compound that when added to cultured cells forms a formazan product that is directly proportional to the number of viable cells. First, 7 mL of SPL1, SPL3, SPL9, SP11, and SPL28 polyphenol extracts were evaporated under nitrogen gas. The solids were re-dissolved to a concentration of 300 mg/mL DMSO (dimethyl sulfoxide) with the aid of a Branson 3510 Ultrasonic Cleaner (Branson Ultrasonics, Danbury, CT). Caco-2 cells were then seeded at a density of 4.0 x 10^4 cells/mL 10% FBS WMEM (working media) in a 96-well microplate for 24 hours. The cells were then treated with 100 µL 75, 150, 300, and 600 µg SPL/mL working media (0.2% DMSO) and incubated at 37°C, 5% CO_2 for 24 hours. After washing twice with working media, 100 µL of media, and 20 µL of CellTiter 96® AQueous One Solution Cell Proliferation Assay (Promega Corporation, Madison, WI, USA) were added to each well. The microplate was incubated for 2 hours at 37° C and 5% CO_2, and absorbance was measured at 490 nm using the microplate reader (Synergy HT Multi-Mode Microplate Reader, BioTek Instruments, Inc. Winooski, VT). Fresh culture media was used as the blank and 0.2% DMSO in media was used as positive control. The average absorbance of the negative control was set at 100% cell viability and the positive control and samples were calculated as percentages of the negative control. All samples and standards were measured in at least triplicate.

3.6 Apoptosis Assay

Apoptosis was measured using the Cell Death Detection ELISA (Enzyme-linked immunosorbent assay) kit (Sigma-Aldrich, St. Louis, MO) by the specific determination of

mono- and oligonucleosomes in the cytoplasmic fraction of Caco-2 cell lysates. 1 x 10^5 cells/mL working media were seeded in 6-well plates and incubated at 37 °C, 5% CO_2 for 24 hours. After incubation, the cells were treated with 2 mL of 600 μg/mL (0.2% DMSO) for 24 hours at 37 °C and 5% CO_2. Treatment media was removed and added to 15 mL centrifuge tubes. Then, cells were incubated at 37 °C and 5% CO_2 with 570 μL of trypsin-EDTA for seven minutes.1.5 mL of EBSS (Earle's Balanced Saline Solution) was added and the full amount was transferred. After centrifuging at 2000 rpm for 5 minutes, the pellet was resuspended in 1 mL media. 100 μL of cells, 500 μL EBBS, and 400 μL Tryptan Blue dye were mixed to count cells in a hemocytometer. After counting, cells were centrifuged at 1,500 x g for 5 minutes and the pellets were resuspended in 500 μL incubation buffer (bottle 5 of Cell Death Detection ELISA kit) per 2 x 10^5 cells. After 30 minutes of incubation at room temperature, the lysate was centrifuged at 18,000 x g for 12 minutes and the amounts of mono- and oligonucleosomes in the supernatants (cytoplasmic fractions) were quantified.

3.7 Statistical Analysis

All statistical analyses were performed using JMP Pro 13.1.0 (SAS Institute Inc., Cary, NC). Data were expressed as mean ± standard deviation (SD) for HPLC analysis and total phenolic and antioxidant capacity assays and as mean ± SEM (standard error of the mean) for cell studies. All experiments were conducted in at least triplicate and significant differences between groups were determined using a Student's t-test at a 5% level of significance ($P < 0.05$). According to the Student's t-test, groups are not significantly different if they share a letter. To compare year and variation (only variations shared in both years) interactions in SPL antioxidant capacity and total polyphenols, fit of least squares models with Tukey's t-test ($p < 0.05$) were used. Total polyphenol and antioxidant capacity correlation among all varieties was also checked.

4. Results

4.1 Year and Variety Comparison of SPL Polyphenol Content and Antioxidant Capacity

To determine the SPL varieties with the highest polyphenol contents and antioxidant capacity, we compared the year impact between 2018 and 2019 (Table 1 and Table 2). Both the range minimum, range maximum, and the average for both total polyphenol content and antioxidant capacity were higher in 2019 SPL than in 2018 SPL (Table 1). This can be attributed to improvements in environmental conditions cultivation of 2019 SPL. The 2018 SPL varieties with the highest total polyphenol contents were SPL20>SPL24>SPL26=SPL21>SPL32 ($p < 0.05$) (Table 2). The same varieties also had the highest antioxidant capacity, but SPL20=SPL24, and SPL21=SPL32 were not significantly different. The 2019 SPL varieties with the highest total polyphenol contents were SPL1>SPL3>SPL9>SPL11=SPL28=SPL7=SPL10=SPL38=SPL32, and with the highest antioxidant capacity were SPL1>SPL3=SPL9>SPL11=SP28=SPL10 ($p < 0.05$). Interactions between year and variety (only varieties shared in both years) were significantly found ($p < 0.05$) in both antioxidant capacity and total polyphenols. According to Tukey's t-test, the least squares mean was higher in 2019 than in 2018 for both antioxidant capacity and total polyphenols ($p < 0.05$). Last, $R^2 = 0.785$ for the correlation between total polyphenol and antioxidant capacity in both years for all varieties (Figure 3).

4.2 Identification and Quantification of Phytochemicals in Top 2019 SPL Varieties

Because the total polyphenol contents and antioxidant capacities of the 2019 SPL varieties were higher, the top 5 highest 2019 SPL varieties in total polyphenol content and antioxidant capacity were selected to identify and quantity the major bioactive compounds. In Figure 4, 3,5-dicaffeyolqunic acid (3,5-diCQA), 3,4-dicaffeoylquninc acid (3,4-diCQA), and chlorogenic acid

(ChA) were the major peaks in all five SPLs. In Table 3, SPL1=SPL9=SPL11 had the significantly highest amounts of (1) ChA and SPL1 was significantly higher than SPL3=SPL28 ($p < 0.05$). SPL1 had the highest quantity of (2) 3,4-diCQA followed by SPL9=SPL3=SPL11=SPL28, and SPL9 was higher than SPL28 ($p < 0.05$). SPL1=SPL3 had the highest amounts of (3) 3,5-diCQA, and SPL3=SPL11=SPL28 and SPL11=SP28=SPL9 meaning that SPL3>SPL9 (groups do not share a letter). (3) 3,5-diCQA is the compound in the highest amounts in all the samples. (4) Tentative flavonoid (TF), the smallest peak, is a tentative flavonoid because it showed maximum light absorption at 353 nm. SPL1 has the highest amount of TF followed by SPL3=SPL11 and SPL11=SPL9=SPL28, meaning SPL3>SPL9=SPL28. For (5) tentative phenolic acid (TP) (maximum light absorption at 328 nm), SPL28=SPL3=SPL11>SPL9, and SPL3=SPL11=SPL1>SPL9, meaning that SPL28>SPL1>SPL9. For the sum of these 5 compounds, SPL1>SPL3=SPL9=SPL11=SPL28 ($p > 0.05$).

In Figure 5, the major peaks in the top five 2019 SPL samples were lutein, chlorophyll B, and chlorophyll A. In Table 4, there was no significant difference between chlorophyll B and chlorophyll A contents in all samples. For lutein, the only statistical difference was SPL28>SPL11. For the sum of lutein and the two chlorophyll compounds, there was no statistical difference between samples.

4.3 Effect of 2019 SPL Polyphenols on Caco-2 Cell Viability

Due to the greater antioxidant capacity and polyphenol content of five 2019 SPL, their anti-colon cancer effects were tested in a Caco-2 colon cancer line model. The positive control, 0.2% dimethyl sulfoxide (DMSO) in working media, was not statistically different compared to the negative control (no treatment), meaning DMSO was not toxic to the cells at 0.2% concentration

(Figure 6). All samples tended to dose-dependently decrease cell viability. Although SPL9, SPL11, and SPL28 did not have the highest antioxidant capacity (Table 2), a total polyphenol (Table 2), or sum of major polyphenols (Table 3), they had the most statistically decreasing effect on Caco-2 cell viability at the two highest concentrations, 300 μg/mL and 600 μg/mL ($p < 0.05$). At 75 μg/mL, in inhibition of % cell viability, SPL28=SPL11>SPL3=SPL1, SPL11=SPL9, SPL9=SPL3=SPL1. At 150 μg/mL, SPL28>SPL11=SPL9>SPL3=SPL1 (Figure 6).

4.4 Effect of 2019 SPL Polyphenols on Caco-2 Cell Apoptosis

Because the top five 2019 SPL tended to dose-dependently decrease cell viability, apoptosis as the mechanism of the effect was investigated. In apoptosis, an endogenous Ca^{+2} and Mg^{+2} -dependent endonuclease is activated that cleaves double-stranded DNA at the internucleosomal linker region, resulting in mono- and oligonucleosomes, which can be detected by enzyme-linked immunosorbent assay (ELISA). The degree of apoptosis was expressed in enrichment factors for the five 2019 SPL samples at 600 μg/mL (Figure 7), which induced a significant decrease in cell viability (Figure 6). All 5 SPL samples increased Caco-2 apoptosis compared to the negative control ($p < 0.05$). SPL28 significantly increased apoptosis compared to SPL1, SPL 3, and SPL 11 ($p < 0.05$).

5. Discussion

Depending on environmental conditions and harvesting period, polyphenol contents and antioxidant capacities of SPLs can vary based on variety and year (Krochmal-Marczak et al., 2020; Suárez et al., 2020). Li et al., (2017) found that the phenolic acid contents across 14 SPL cultivars were significantly different. Therefore, in the present study, the interannual and inter-variation differences in polyphenol content and antioxidant capacity of SPLs were first

compared. 2019 SPL varieties possessed higher average antioxidant capacity and polyphenol content than the 2018 SPL varieties. This can be explained by differences in environmental conditions such as an increase in stress from sunlight. In the present study, the average polyphenol content in 2019 SPLs was 53 ± 9 mg gallic acid equivalents/g dry weight (DW). In comparison, Islam et al. (2002) found an average of 60 mg polyphenols/g DW SPL and Sun et al., (2014) found 30-120 mg polyphenols /g DW SPL. Our SPL had higher total polyphenol content in comparison to the total content of polyphenols in spinach samples, which ranged from 1.8 to 5 mg polyphenols /g of dry matter (Ligor et al., 2013) and in kale samples, where 3 g polyphenols / g dry matter were found (Ligor et al., 2013).

In the present study, 2019 SPL1, SPL3, SPL9, SPL11, SPL28 were the varieties with the highest antioxidant capacity and polyphenol content (Table 2) and were therefore selected for major polyphenol characterization and anti-colon cancer assays. Studies have found that fruits and vegetables with high antioxidant capacity also possess health-promoting properties, such as anti-cancer effects (Zhang *et al.,* 2015; Boivin *et al.,* 2009). Not many studies have evaluated the health-promoting properties of SPL compounds against diseases such as cancer (Karna *et al.,* 2011; Kurata *et al.,* 2007; Vishnu *et al.,* 2019). Cancer is the second leading cause of death in the United States and it is a main public health issue globally (Siegel *et al.,* 2016). Specifically, colorectal cancer, cancer of both the colon and rectum, is the third most common malignancy and the fourth leading cause of cancer-related deaths worldwide (Favoriti *et al.,* 2016). Even using aggressive therapies, tumors are resistant to modern treatment protocols (Rosa *et al.,* 2018). Epidemiological data have suggested that diet is the main factor in colorectal cancer etiology (Rosa *et al.,* 2018).

Caco-2 cells are a good model of cancer of the enterocytes of the small and large intestine as they express many of the enzymes and transporters of primary epithelial cells (Verhoeckx *et al.,* 2015). As far as it is known, this is the first study to have investigated the anti-colon cancer effect of sweetpotato leaf (SPL) polyphenols on Caco-2 cells. In Kurata *et al.* (2007), caffeic acid standard and di- and tri-caffeoylquinic acids purified from SPLs collectively dose-dependently depressed cancer cell proliferation at concentrations between 10-1000 µM in three cancer cell lines: stomach (Kato-III), colon (DLD-1), and leukemia (HL-60). 3,4,5-tri-*O*-caffeolylquinic acid was most effective in depressing the growth of all three cancer cell lines. In the present study, SPL extract in concentrations of 75, 150, 300, and 600 µg/mL were administered to the Caco-2 cell line to measure cell viability and apoptosis. Caco-2 cells have been shown to mimic enterocytic features better than DLD-1 cells under standard culture conditions (Lelièvre *et al.,* 1998). Vishnu *et al*. (2019) found that SPLs possessed anti-colon cancer properties against HCT-116 colon cancer cells at concentrations of 100, 200, and 400 µg/mL. However, Vishnu *et al.* (2019) used purple sweetpotato leaf anthocyanins, instead of polyphenols as in the current study, as the treatment. In Taira *et al.* (2013), SPL extracts at 0.22, 1.10, and 2.20 mg/mL and individual phenolic acid compounds at 12.5, 25, and 50 µM were found to modulate the downstream Wnt signaling pathway and to decrease cell viability in HCT-116 cells, suggesting that sweetpotato leaves may be a protective food against colon cancer.

Proliferation is a key part of cancer development and progression in many cancers, including colon (Feitelson *et al.,* 2015). Several naturally occurring phytochemicals, including curcumin, resveratrol, epigallocatechin-3-gallate, lycopene, and quercetin have shown to inhibit one or more pathways that contribute to proliferation (Feitelson *et al.,* 2015). Polyphenols such as ferulic acid, *p*-coumaric acid, and quercetin have been reported to decrease proliferation in colon

cancer cell lines HCT-15 (Roy *et al.,* 2016) and Caco-2 (Zhou *et al.,* 2019). In the present study, SPL extracts containing chlorogenic acid, 3,4-dicaffeoylquinic acid, and 3,5-dicaffeoylquinic acid significantly inhibited Caco-2 cell proliferation in a dose-dependent manner.

In general, cancer cell death can be classified as either necrosis or apoptosis. Necrosis is the unprogrammed, or accidental, death of cells and living tissues (Zhang *et al.,* 2018). On the other hand, apoptosis is programmed cell death. It is the most common form of eukaryotic cell death (Bunel *et al.*, 2013). In the present study, SPL extracts from 5 varieties significantly increased Caco-2 cell apoptosis compared to the negative control. Karna *et al.* (2011) demonstrated that SPLs were able to decrease proliferation and increase apoptosis in PC-3 prostate cancer cells in *vitro* and tumor xenografts of human prostate cancer in nude mice. Phenolics acids from blueberry extract were also reported to induce apoptosis in Caco-2 cell lines (Yi *et al.* 2005).

6. Conclusions

Compared to 2018 SPL extracts, SPL extracts harvested in 2019 showed higher polyphenol contents and antioxidant activity. The 2019 SPL varieties SPL1, SPL3, SPL9, SPL11, SPL28 ranked the highest in total polyphenol content and antioxidant capacity. The major polyphenols identified were chlorogenic acid, 3,4-dicaffeoylqunic acid (3,4-diCQA), and 3,5-dicaffeoylquninc acid (3,5-diCQA). 3,5-diCQA was present in the highest amounts for all samples and SPL1 was the highest in the sum of the major compounds. All top five 2019 SPL samples significantly decreased Caco-2 cell proliferation in a dose-dependent manner and significantly increased apoptosis as compared to the control. These findings suggest that there are significant differences in polyphenol content and antioxidant capacity between year and among genotypes. Furthermore, SPL polyphenols can decrease colon cancer cell proliferation by increasing apoptosis in a Caco-2 cell model. Further studies in animal models are warranted.

References

Akkari, H., Hajaji, S., B'chir, F., Rekik, M., & Gharbi, M. (2016). Correlation of polyphenolic content with radical-scavenging capacity and anthelmintic effects of Rubus ulmifolius (Rosaceae) against Haemonchus contortus. *Veterinary Parasitology*, *221*, 46–53.

Boivin, D., Lamy, S., Lord-Dufour, S., Jackson, J., Beaulieu, E., Côté, M., Moghrabi, A., et al. (2009). Antiproliferative and antioxidant activities of common vegetables: A comparative study. *Food Chemistry*, *112*(2), 374–380.

Bunel, V., Ouedraogo, M., Nguyen, A., Stévigny, C., & Duez, P. (2014). Methods Applied to the In Vitro Primary Toxicology Testing of Natural Products: State of the Art, Strengths, and Limits. *Planta Medica*, *80*(14), 1210–1226.

Favoriti, P., Carbone, G., Greco, M., Pirozzi, F., Pirozzi, R. E. M., & Corcione, F. (2016). Worldwide burden of colorectal cancer: A review. *Updates in Surgery*, *68*(1), 7–11.

Feitelson, M. A., Arzumanyan, A., Kulathinal, R. J., Blain, S. W., Holcombe, R. F., Mahajna, J., Marino, M., et al. (2015). Sustained proliferation in cancer: Mechanisms and novel therapeutic targets. *Seminars in Cancer Biology*, *35*, S25–S54.

Hue et al. (2012). Antioxidant activity, phenolic and flavonoid contents in the leaves of different varieties of sweet potato (Ipomoea batatas). *Australian Journal of Crop Science*, *6*(3), 375–380.

Islam, Md. S., Yoshimoto, M., Yahara, S., Okuno, S., Ishiguro, K., & Yamakawa, O. (2002). Identification and Characterization of Foliar Polyphenolic Composition in Sweetpotato (*Ipomoea batatas* L.) Genotypes. *Journal of Agricultural and Food Chemistry*, *50*(13), 3718–3722.

Karna, P., Gundala, S. R., Gupta, M. V., Shamsi, S. A., Pace, R. D., Yates, C., Narayan, S., et al. (2011). Polyphenol-rich sweet potato greens extract inhibits proliferation and induces apoptosis in prostate cancer cells in vitro and in vivo. *Carcinogenesis*, *32*(12), 1872–1880.

Krochmal-Marczak, B., Cebulak, T., Kapusta, I., Oszmiański, J., Kaszuba, J., & Żurek, N. (2020). The Content of Phenolic Acids and Flavonols in the Leaves of Nine Varieties of Sweet Potatoes (Ipomoea batatas L.) Depending on Their Development, Grown in Central Europe. *Molecules*, *25*(15), 3473.

Kurata, R., Adachi, M., Yamakawa, O., & Yoshimoto, M. (2007). Growth Suppression of Human Cancer Cells by Polyphenolics from Sweetpotato (*Ipomoea batatas* L.) Leaves. *Journal of Agricultural and Food Chemistry*, *55*(1), 185–190.

Lelièvre, V., Muller, J.-M., & Falcòn, J. (1998). Adenosine modulates cell proliferation in human colonic carcinoma. II. Differential behavior of HT29, DLD-1, Caco-2 and SW403 cell lines. *European Journal of Pharmacology*, *341*(2–3), 299–308.

Li, M., Jang, G. Y., Lee, S. H., Kim, M. Y., Hwang, S. G., Sin, H. M., Kim, H. S., et al. (2017). Comparison of functional components in various sweet potato leaves and stalks. *Food Science and Biotechnology*, *26*(1), 97–103.

Ligor, M., Trziszka, T., & Buszewski, B. (2013). Study of Antioxidant Activity of Biologically Active Compounds Isolated from Green Vegetables by Coupled Analytical Techniques. *Food Analytical Methods*, *6*(2), 630–636.

Rosa, L., Jordão, N., da Costa Pereira Soares, N., deMesquita, J., Monteiro, M., & Teodoro, A. (2018). Pharmacokinetic, Antiproliferative and Apoptotic Effects of Phenolic Acids in Human Colon Adenocarcinoma Cells Using In Vitro and In Silico Approaches. *Molecules*, *23*(10), 2569.

Roy et al. (2016). Plant Phenolics Ferulic Acid and P-Coumaric Acid Inhibit Colorectal Cancer Cell Proliferation through EGFR Down- Regulation. *Asian Pacific Journal of Cancer Prevention*, *17*, 8. APJCP.

Siegel, R. L., Miller, K. D., & Jemal, A. (2016). Cancer statistics, 2016: Cancer Statistics, 2016. *CA: A Cancer Journal for Clinicians*, *66*(1), 7–30.

Slinkard, K., & Singleton, V. L. (1977). Total Phenol Analysis: Automation and Comparison with Manual Methods. *American Journal of Enology and Viticulture*, *28*(1), 49.

Suárez, S., Mu, T., Sun, H., & Añón, M. C. (2020). Antioxidant activity, nutritional, and phenolic composition of sweet potato leaves as affected by harvesting period. *International Journal of Food Properties*, *23*(1), 178–188.

Sun, H., Mu, T., Xi, L., Zhang, M., & Chen, J. (2014). Sweet potato (Ipomoea batatas L.) leaves as nutritional and functional foods. *Food Chemistry*, *156*, 380–389.

Taira, J., Uehara, M., Tsuchida, E., & Ohmine, W. (2014). Inhibition of the β-Catenin/Tcf Signaling by Caffeoylquinic Acids in Sweet Potato Leaf through down Regulation of the Tcf-4 Transcription. *Journal of Agricultural and Food Chemistry*, *62*(1), 167–172.

Verhoeckx, K., Cotter, P., López-Expósito, I., Kleiveland, C., Lea, T., Mackie, A., Requena, T., et al. (Eds.). (2015). *The Impact of Food Bioactives on Health*. Cham: Springer International Publishing. Retrieved October 17, 2020, from http://link.springer.com/10.1007/978-3-319-16104-4

Vishnu, V. R., Renjith, R. S., Mukherjee, A., Anil, S. R., Sreekumar, J., & Jyothi, A. N. (2019). Comparative Study on the Chemical Structure and *In Vitro* Antiproliferative Activity of Anthocyanins in Purple Root Tubers and Leaves of Sweet Potato (*Ipomoea batatas*). *Journal of Agricultural and Food Chemistry*, *67*(9), 2467–2475.

Yi, W., Fischer, J., Krewer, G., & Akoh, C. C. (2005). Phenolic Compounds from Blueberries Can Inhibit Colon Cancer Cell Proliferation and Induce Apoptosis. *Journal of Agricultural and Food Chemistry*, *53*(18), 7320–7329.

Zhang, Y., Chen, X., Gueydan, C., & Han, J. (2018). Plasma membrane changes during programmed cell deaths. *Cell Research*, *28*(1), 9–21.

Zhang, Y.-J., Gan, R.-Y., Li, S., Zhou, Y., Li, A.-N., Xu, D.-P., & Li, H.-B. (2015). Antioxidant Phytochemicals for the Prevention and Treatment of Chronic Diseases. *Molecules*, *20*(12), 21138–21156.

Zhou, W., Liang, X., Dai, P., Chen, Y., Zhang, Y., Zhang, M., Lu, L., et al. (2019). Alteration of Phenolic Composition in Lettuce (Lactuca sativa L.) by Reducing Nitrogen Supply Enhances its Anti-Proliferative Effects on Colorectal Cancer Cells. *International Journal of Molecular Sciences*, *20*(17), 4205.

Table 1. Summary of 2018 and 2019 SPL Total Polyphenols and Antioxidant Capacity

	2018 SPL n = 3	**2019 SPL** n = 3
Total Polyphenols (mg gallic acid equivalents/g)	43 ± 13 (24-72)	53 ± 9 (38-84)
Antioxidant Capacity (µmol Trolox equivalents/g)	110 ± 75 (18-287)	132 ± 32 (73-236)

Data expressed as mean ± SD (range of data). 2018 (27 SPL) & 2019 (24 SPL), n = number of replications, $p < 0.05$

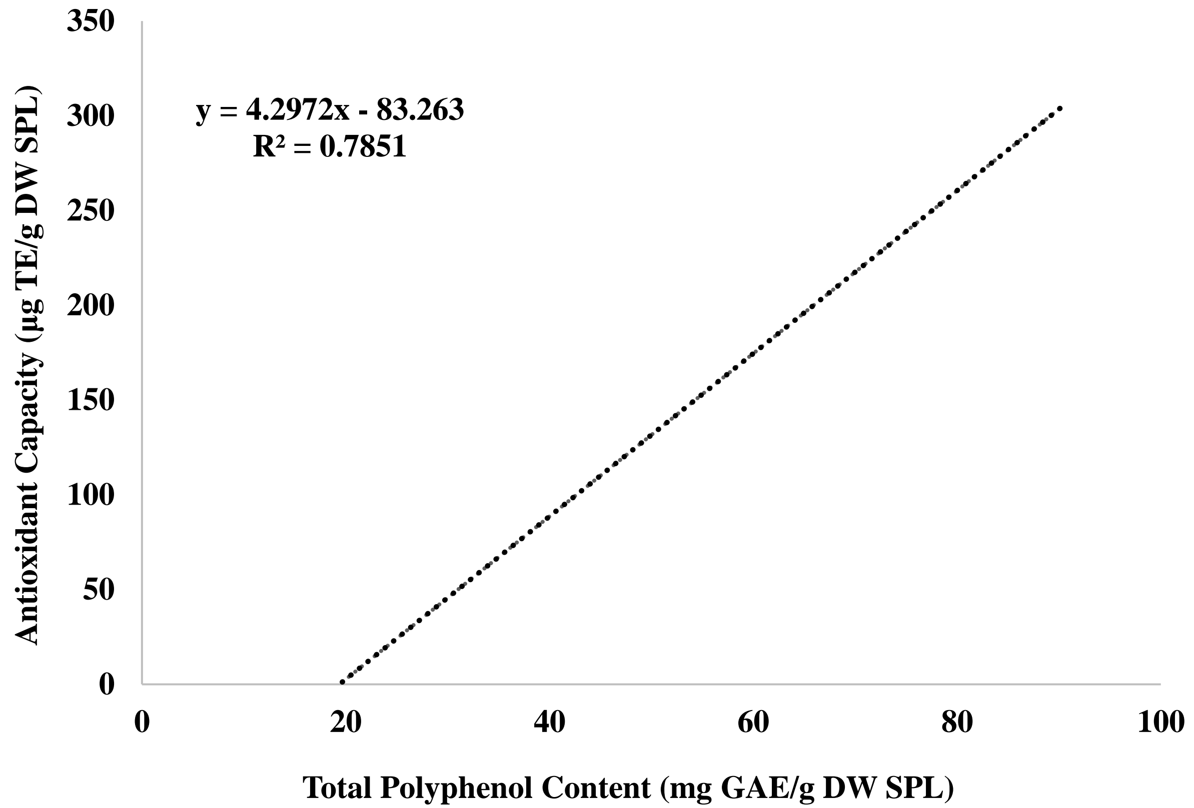

Figure 3. Correlation of 2018 and 2019 SPL Antioxidant Capacities and Total Polyphenol Contents in All Varieties. GAE = Gallic Acid Equivalents, TE = Trolox Equivalents.

Table 2. Total Polyphenols and Antioxidant Capacity in SPL harvested in 2018 and 2019

SPLs	Total Polyphenols (mg gallic acid Equiv/g)		Antioxidant Capacity (µmol Trolox Equiv/g)	
	2018	2019	2018	2019
#1	61.1 ± 6.4 C	83.7 ± 6.7 A	155.3 ± 15.6 DE	236.4 ± 20.0 A
#3	44.0 ± 3.6 EFG	65.1 ± 3.4 B	74.8 ± 15.1 GH	162.6 ± 19.0 BC
#5	43.1 ± 2.7 FGH	-	84.1 ± 10.1 G	-
#7	45.5 ± 2.6 EF	55.9 ± 4.0 DEF	81.6 ± 14.6 GH	133.0 ± 21.2 E
#8	36.2 ± 4.0 JK	-	55.7 ± 12.1 IJ	-
#9	41.2 ± 1.3 GHI	60.6 ± 4.9 C	65.0 ± 9.1 HI	168.4 ± 23.0 B
#10	32.0 ± 2.3 L	56.8 ± 5.6 D	39.4 ± 8.3 JK	150.0 ± 16.1 CD
#11	42.8 ± 2.1 FGH	55.7 ± 3.3 DEF	81.3 ± 9.3 GH	151.9 ± 15.3 CD
#15	25.7 ± 3.2 M	49.7 ± 2.3 G	25.3 ± 10.6 KL	126.5 ± 17.5 EF
#16	47.0 ± 2.6 E	50.0 ± 3.2 G	104.4 ± 24.8 F	128.2 ± 29.1 EF
#18	59.4 ± 1.2 C	54.3 ± 3.6 F	172.8 ± 42.9 CD	133.9 ± 18.1 E
#19	45.6 ± 3.0 EF	50.0 ± 2.5 G	139.8 ± 10.3 E	128.7 ± 18.4 EF
#20	72 ± 0.7 A	-	286.5 ± 7.7 A	-
#21	50.7 ± 2.0 C	47.2 ± 3.5 HI	182.3 ± 14.1 C	122.9 ± 20.4 EF
#23	40.8 ± 3.4 HI	45.7 ± 2.5 IJK	117.0 ± 7.9 F	123.0 ± 15.8 EF
#24	67.8 ± 4.1 B	42.6 ± 3.0 IJK	276.2 ± 33.8 A	118.5 ± 16.0 F
#26	60.3 ± 2.0 C	46.8 ± 3.1 IJ	235.5 ± 10.2 B	129.7 ± 18.7 EF
#28	-	56.6 ± 2.5 DEF	-	154.6 ± 22.7 CD
#32	52.0 ± 1.8 D	55.9 ± 4.0 DEF	178.3 ± 8.7 C	147.6 ± 26.4 E
#34	52.4 ± 3.3 D	54.4 ± 5.3 EF	173.1 ± 11.3 CD	127.1 ± 27.5 EF
#35	34.4 ± 1.7 KL	44.6 ± 3.3 JK	77.8 ± 5.3 GH	84.1 ± 19.7 H
#36	21.7 ± 2.3 N	49.3 ± 2.8 GH	20.9 ± 4.3 L	102.4 ± 24.4 G
#37	25.2 ± 1.2 M	-	21.3 ± 12.4 L	-
#38	42.8 ± 1.6 FGH	57.1 ± 3.4 D	116.3 ± 17.1 F	132.8 ± 20.9 E
#39	38.5 ± 3.0 IJ	43.9 ± 2.6 KL	81.3 ± 9.3 GH	98.5 ± 27.4 G
#40	36.9 ± 1.3 JK	54.7 ± 3.6 EF	53.2 ± 9.0 IJ	131.2 ± 27.0 EF
#41	23.0 ± 0.9 MN	-	40.8 ± 12.2 JK	-
#44	-	44.8 ± 1.9 JK	-	98.3 ± 23.4 G
#45	24.1 ± 0.9 MN	37.6 ± 1.4 M	18.0 ± 5.8 L	73.5 ± 21.8 H

Data expressed as mean ± SD (n =3). Equiv=equivalent. (-) = no sample. Values within columns with different letters are significantly different ($p < 0.05$).

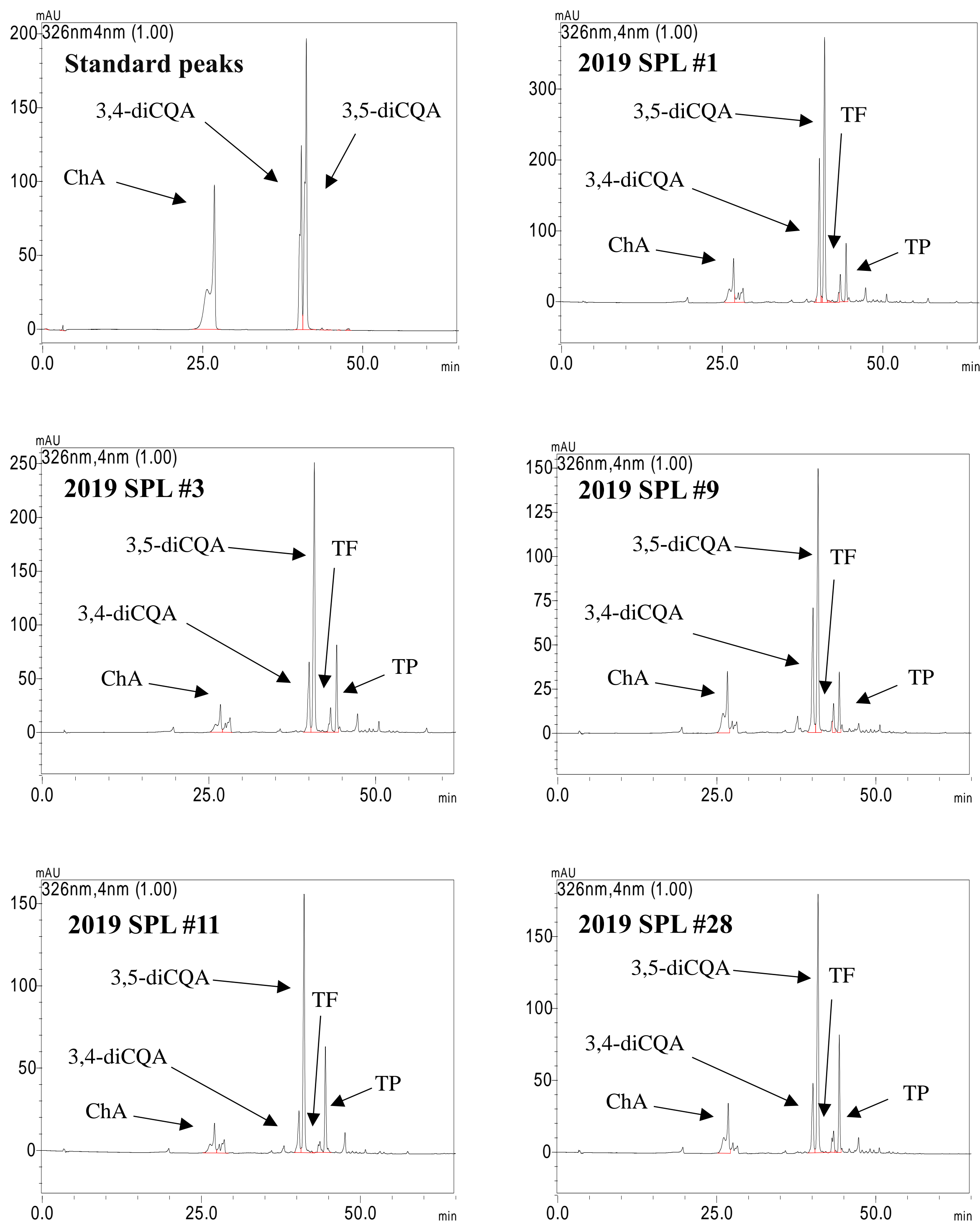

Figure 4. HPLC Chromatograms of Polyenols in Five 2019 SPL. ChA = chlorogenic acid, 3,4-diCQA = 3,4-dicaffeoylquinic acid, 3,5-diCQA = 3,5- dicaffeoylqunic acid, TF = tentative flavonoid (absorption at 353 nm), TP = tentative phenolic (absorption at 328 nm)

Table 3. Concentrations of Polyphenols in Five SPLs harvested in 2019

Compound	Amount (mg/g)				
	SPL1	**SPL3**	**SPL9**	**SPL11**	**SPL28**
Chlorogenic acid	17.9 ± 3.3 [a]	9.3 ± 0.2 [b]	13.4 ± 3.7 [ab]	13.1 ± 1.9 [ab]	8.4 ± 1.4 [b]
3,4-dicaffeoylqunic acid	51.3 ± 9.5 [a]	22.0 ± 0.6 [bc]	24.3 ± 6.2 [b]	16.2 ± 2.4 [bc]	11.7 ± 2.1 [c]
3,5-dicaffeoylquinic acid	58.5 ± 11.4 [a]	51.4 ± 1.4 [ab]	31.7 ± 9.0 [c]	37.3 ± 5.8 [bc]	40.5 ± 7.4 [bc]
Tentative flavonoid	5.7 ± 0.6 [a]	4.4 ± 0.7 [b]	3.1 ± 0.5 [c]	3.6 ± 0.2 [bc]	2.6 ± 0.6 [c]
Tentative phenolic	9.8 ± 1.9 [b]	12.4 ± 0.2 [ab]	5.4 ± 1.1 [c]	12.9 ± 1.9 [ab]	14.1 ± 2.6 [a]
Total	**143.7 ± 26.7 [a]**	**99.6 ± 3.1 [b]**	**77.8 ± 20.5 [b]**	**83.0 ± 12.2 [b]**	**77.3 ± 14.1 [b]**

Data represented as mean ± SD (mg/g) for peaks 1 - 3 and mean ± SD (mg chlorogenic acid equivalents/g) for peaks 4-5 (n = 3). Peak 4 λ_{max} is at 353 nm (tentative flavonoid) and peak 5 λ_{max} is at 328 nm (tentative phenolic acid). Means followed by a common letter are not significantly different by the Student's t-test at 5% level of significance.

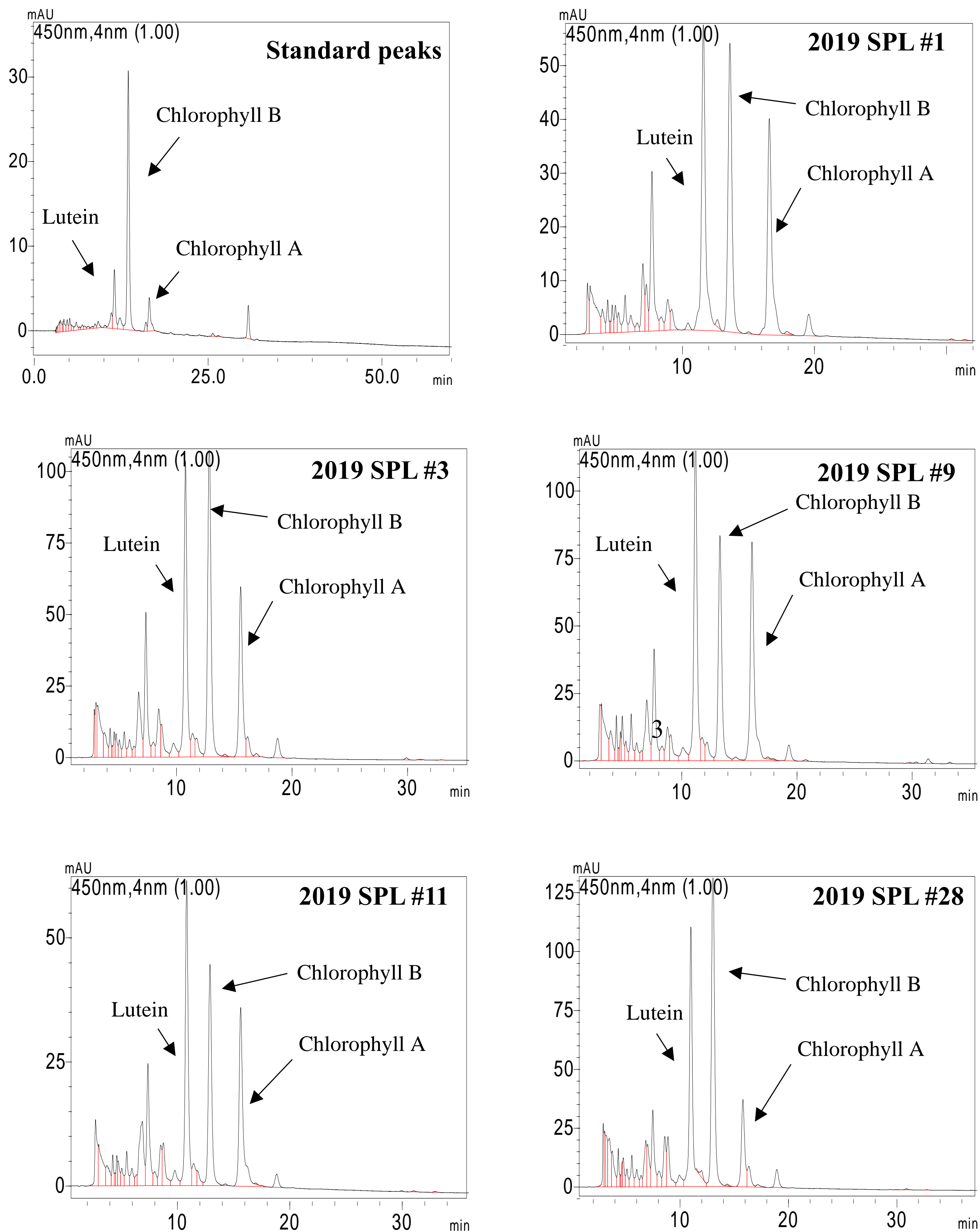

Figure 5. HPLC Chromatograms of Carotenoids and Chlorophylls in Five 2019 SPL.

Table 4. Concentrations of Chlorophyll and Carotenoid Compounds in Five 2019 SPL

Compound	Amount (mg/g)				
	SPL1	SPL3	SPL9	SPL11	SPL28
Lutein	0.18 ± 0.07 [ab]	0.21 ± 0.03 [ab]	0.19 ± 0.01 [ab]	0.13 ± 0.04 [b]	0.31 ± 0.01 [a]
Chlorophyll B	0.39 ± 0.01 [a]	0.50 ± 0.12 [a]	0.63 ± 0.15 [a]	0.40 ± 0.03 [a]	0.64 ± 0.11 [a]
Chlorophyll A	0.18 ± 0.08 [a]	0.23 ± 0.12 [a]	0.35 ± 0.20 [a]	0.22 ± 0.03 [a]	0.16 ± 0.06 [a]
Total	**0.75 ± 0.16 [a]**	**0.94 ± 0.27 [a]**	**1.17 ± 0.36 [a]**	**0.75 ± 0.10 [a]**	**1.11 ± 0.18 [a]**

Data are represented as the mean ± SD (n = 3). Values within columns with different letters are significantly different ($p < 0.05$).

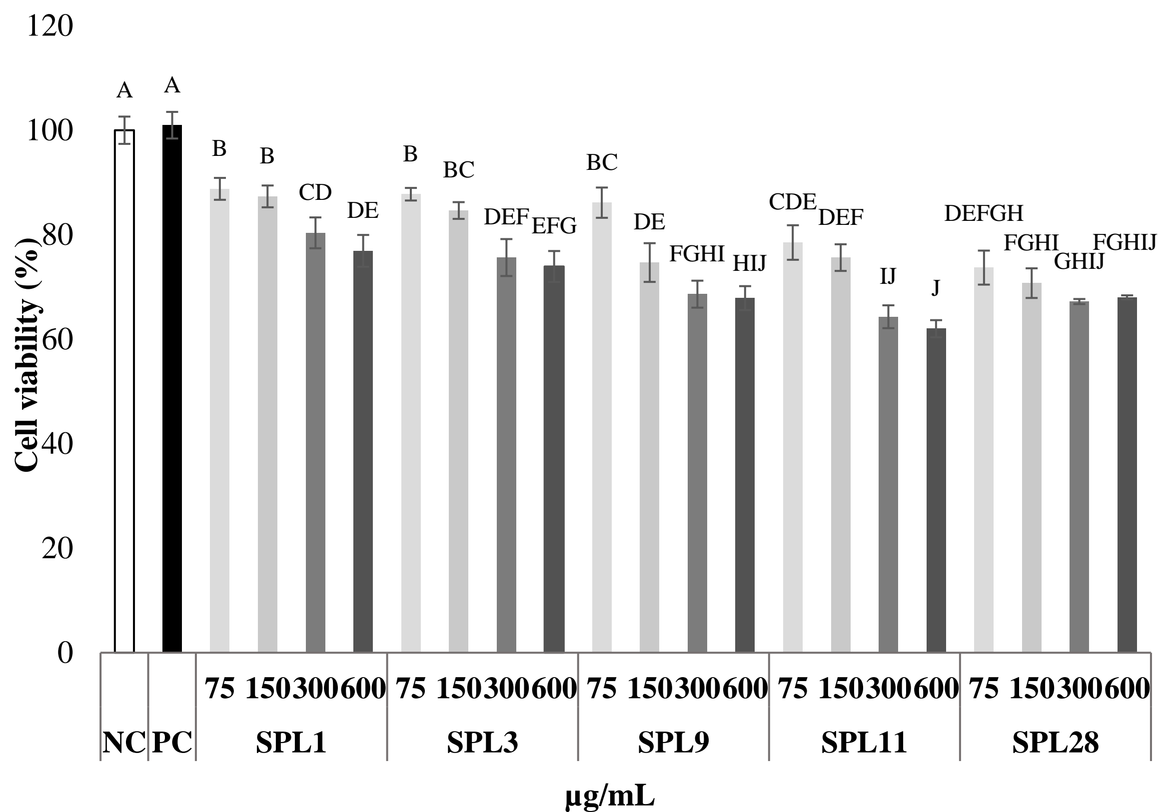

Figure 6. Effect of Five 2019 SPL Varieties on Caco-2 Cell Viability. Data are represented as the mean ± SEM (n = 3). Means followed by a common letter are not significantly different by the Student's t-test at a 5% level of significance. NC = negative control (working media), PC = positive control (0.2% DMSO in media).

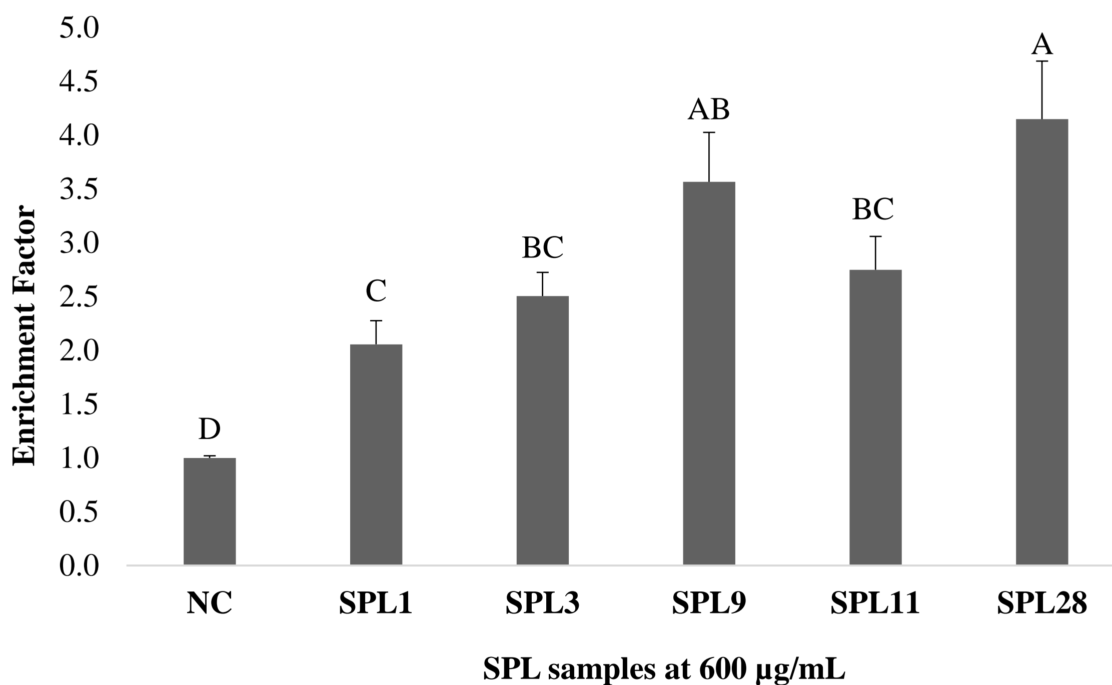

Figure 7. Effect of Five 2019 SPL Varieties on The Apoptosis of Caco-2 Cells. Enrichment factor is the amount of mono- and oligonucleosomes in the cytosol due to apoptosis. Data are represented as the mean ± SEM (n = 3). Means followed by a common letter are not significantly different by the Student's t-test at a 5% level of significance. NC = negative control (working media).

Overall Conclusions

In this study, the antioxidant capacity and polyphenol content of sweetpotato leaves (SPL) differed based on year and variety. The 2019 SPL varieties SPL1, SPL3, SPL9, SPL11, and SPL28, having the highest antioxidant capacity and polyphenol content, were selected for further studies. Chlorogenic acid, 3,4-dicaffeoylquninic acid (3,4-diCQA), and 3,5-dicaffeoylquinic acid (3,5-diCQA) were the major polyphenols identified, and SPL1 had the highest amounts. All top 5 2019 SPL samples decreased Caco-2 proliferation in a dose-dependent manner and increased Caco-2 apoptosis at dose 600 µg/mL. SPL11 was the most effective in decreasing cell viability while SPL28 was most effective in increasing apoptosis. Several other researchers have reported that SPLs are a superior source of antioxidant polyphenolics compared to other commercial vegetables. In addition, these findings suggest that SPLs may have potential effects against colorectal cancer, and further studies are imperative to further investigate if the leaves of the sweetpotato crop can provide a solution for the resource and health problems of the 21st century.

Appendix

Office of Research Compliance

March 14, 2019

MEMORANDUM

TO: Dr. Sun-Ok Lee

FROM: Ines Pinto, Biosafety Committee Chair

RE: Protocol Modification

PROTOCOL #: 16011

PROTOCOL TITLE: Testing bioactive components (arachidins, sweet potato extract, conjugated linoleic acid, saponins, berry volatiles/phenolics) for proliferation and inflammatory responses

APPROVED PROJECT PERIOD: **Start Date** September 10, 2015 **Expiration Date** September 9, 2021

The Institutional Biosafety Committee (IBC) has approved your request, dated March 8, 2019, to modify Protocol # 16011, "Testing bioactive components (arachidins, sweet potato extract, conjugated linoleic acid, saponins, berry volatiles/phenolics) for proliferation and inflammatory responses" to add laboratory personnel.

The IBC appreciates your assistance and cooperation in complying with University and Federal guidelines for research involving hazardous biological materials.

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