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# Antioxidant Activities and Phenolic Acids in Different Raw and Boiled Potatoes and Sweet Potatoes

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ANTIOXIDANT ACTIVITIES AND PHENOLIC ACIDS IN DIFFERENT RAW  
AND BOILED POTATOES AND SWEET POTATOES

A Thesis

Submitted to the Graduate Faculty of the  
Louisiana State University and  
Agricultural and Mechanical College  
in partial fulfillment of the  
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Master of Science

in

The School of Nutrition and Food Sciences

by

Ya Gao

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## ABSTRACT

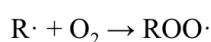
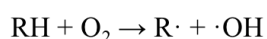
Antioxidants have attracted great interests from food industries and scientists in recent years, because they have the capacity to reduce the harmful oxidation reaction in human body. Phenolic compounds are the common antioxidants that are widely found in fruits and vegetables. In this study, three varieties of potatoes and four varieties of sweet potatoes were used to examine the profiles and contents of phenolics in the raw and boiled potatoes and sweet potatoes. The Red Garnet Sweet Potato (RGSP) was the richest in phenolic content and selected to study the changes of free phenolics, total phenolic content, and antioxidant activity at different boiling times. In general, gallic acid and six different chlorogenic acid derivatives were found in the potatoes and sweet potatoes. The varieties of phenolic acids in the sweet potatoes were much more than the potatoes. The content of each phenolic acid varied in different raw potatoes and sweet potatoes. The total phenolics content of raw RGSP was the highest among these samples. A short time of boiling processing for potatoes and sweet potatoes could cause a significant increase in free phenolic acids, except 3-O-caffeoylquinic acid and 3,4-O-dicaffeoylquinic acid which were found to decrease during the processing. After boiled for 10 min, RGSP had higher level of phenolics and antioxidant activity than its raw or the one boiled for longer time. Therefore, the level of free phenolics and antioxidant activity in potatoes and sweet potatoes changed during boiling. They generally reached the peak level in a short boiling time (10 min) and then decreased with extended boiling time.

## CHAPTER 1. LITERATURE REVIEW

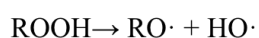
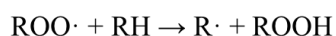
### 1.1 Lipid Oxidation

Lipid oxidation is a molecular-based reaction which could damage cell structure and result in cell inflammatory in either plants or animals (Repetto et al., 2012). Generally, lipid oxidation could be initiated via all kinds of oxidant pathways and affected by different internal or external factors. For example, the oxidants include various enzymes, light, a wide range of reactive oxygen species and free radicals or the combination of transition metal ions and peroxides (Pan et al., 2013). The most commonly occurred lipid oxidation is autoxidation that involves in three phases: (1) initiation, the formation of free radicals; (2) propagation, the free radical chain reactions; (3) termination, the formation of non-radical products (Figure 1.1). As a result, a variety of non-radical products are produced in these reactions, such as alcohols, ketone, alkanes, aldehydes and ethers (Dianzani et al., 2008).

#### Initiation:



#### Propagation:



#### Termination:

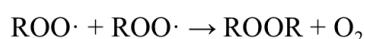
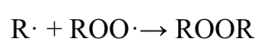
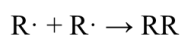


Figure 1.1 Mechanism for lipid autoxidation in different phases. Free radical ( $\text{R}\cdot$ ), hydroperoxide ( $\text{ROOH}$ ), hydroperoxyl radical ( $\text{ROO}\cdot$ ) (Repetto et al., 2012).

Enzymatic oxidation and photosensitized oxidation are another two types of lipid oxidation. In the enzymatic oxidation, lipoxygenases could catalyze free and esterified

fatty acids oxidation and lipoprotein oxidation (Pan et al., 2000). Photosensitized oxidation is initiated by certain radiations or photosensitizers to yield more cyclic products than autoxidation (Neff, 1980).

Lipid oxidation could not only affect the nutritional and flavor quality of foods, but also produce free radicals which are responsible for health problems and accelerated aging (Park et al., 2014; Finley et al., 1993; Aruoma, 1998). In biological systems, the oxidation and reduction reactions represent the basic metabolic changes of biochemical mechanism. However, excessive free radicals would break or disturb the normal metabolism (Pan et al., 2013). Therefore, it is necessary to inhibit lipid oxidation and maintain the quality of food as well as reduce the risks of developing human chronic diseases.

## **1.2 Antioxidant**

In order to maintain food quality and reduce harmful lipid oxidation involved in chronic diseases, antioxidants are used in both food industry and medical supplements. Generally, antioxidant is defined as an exogenous (natural or synthetic) or endogenous compound which can act in many ways, like removing oxygen, scavenging reactive oxygen species or their precursor, inhibiting the reactive oxygen species (ROS) formation and binding desired metal ion to the catalysis of ROS generation (Gilgun-Sherki et al., 2002). Recently, antioxidants have widely been used as the dietary supplements to prevent chronic diseases for human beings. In industrial uses, they can prolong shelf life of the food and cosmetics and prevent degradation of the rubber and gasoline.

### 1.2.1 Synthetic Antioxidants

Synthetic antioxidants include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ), and propyl gallate (PG). However, synthetic antioxidants have carcinogenicity, teratogenicity and acute toxicity. The use of synthetic antioxidant is restricted by legislation (Lingk, 1991; Unnikrishnan et al., 2002). The chemical structures of synthetic antioxidants are showed in Figure 1.2. Their limits of using in food products are showed in Table 1.1

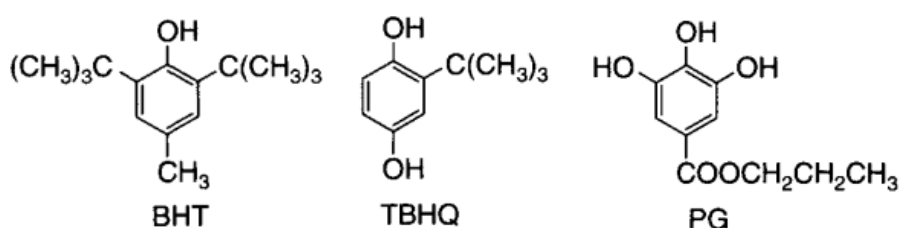


Figure 1.2 Chemical structures of BHT, TBHQ and PG

Table 1.1 FDA limits and tolerances for synthetic antioxidants in select food products \*

Reference	Application	Antioxidant	Limit (percent weight of food unless noted)
21 CFR 182.3169	general	BHA	0.02% singly or in combination of fat or oil portion of food including the essential oil, except where prohibited by Standard of Identity
21 CFR 182.3173		BHT	
21 CFR 172.185		TBHQ	
21 CFR 184.1660		PG	
<b>Specific Foods</b>			
21 CFR 172.615	chewing gum base	BHA, BHT, PG	0.1% singly or in combination
21 CFR 172.515	synthetic flavorings	BHA	0.5% of essential (volatile) oil
21 CFR 172.110	active dry yeast	BHA	0.1%
	beverages and desserts prepared from dry mixes		0.0002%
	dry mixes for beverages and		0.009%
	dry diced glazed fruit		0.0032%

Table 1.1 continued

21 CFR 172.110 and/or 21 CFR 172.115	dry breakfast cereals	BHA and/or BHT	0.005%
	emulsion stabilizers for shortening		0.02%
	potato granules		0.001%
	potato flakes, sweet potato flakes, and dehydrated potato shreds		0.005%
<b>Standardized Foods</b>			
21 CFR 137.350	enriched parboiled rice	BHT	0.0033%
21 CFR 161.175	frozen raw breaded shrimp	BHA, BHT	0.02% of fat or oil content
21 CFR 166.110	margarine	BHA, BHT, PG	0.02% singly or in combination based on finished product
<b>Meat and Poultry Products</b>			
9 CFR 424.21	dry sausage	BHA, BHT, TBHQ, PG <sup>†</sup>	0.003% singly, 0.006% in combination, with no antioxidant exceeding 0.003%
	fresh pork and/or beef sausage, brown- and-serve sausage, pre-grilled beef patties, pizza toppings, and meatballs		0.01% singly, 0.02% in combination, with no antioxidant exceeding 0.01%, based on fat content
	dried meats		0.01% singly, or in combination
	rendered animal fat or a combination of such fat and vegetable fat		0.01% singly, 0.02% in combination, with no antioxidant exceeding
	various poultry products		0.01% singly, 0.02% in combination, with no antioxidant exceeding 0.01% based on fat content

\*Regulations subject to change

<sup>†</sup>TBHQ and PC cannot be used in combination (Source: FDA, 2012)

## 1.2.2 Natural Antioxidants

Free radicals are well known for the high capacity to attack cell molecules in the body and further interfere with the cell structure and affect their functions (Lobo, 2010). However, natural antioxidants are the compounds that could reduce the oxidative attack to the body by scavenging free radicals (Shah et al., 2014). Fruits and vegetables are the good sources of natural antioxidants. A number of studies have reported that antioxidants are found in vegetables (broccoli, potato, sweet potato, drumstick, pumpkin, curry and nettle), fruits (grapes, pomegranate, date and kinnow), grain cereals, eggs, meat, legumes, nuts, herbs and spices (tea, rosemary, oregano, cinnamon, sage, thyme, mint, ginger and clove) (Shah et al., 2014). Some selected antioxidant compounds and their major dietary sources are demonstrated Table 1.2.

Table 1.2 Common antioxidant compounds and their major dietary sources

Group	Name	Major Existing Foods
Vitamin A	Retinoids	Green and yellow vegetables, liver, oily fishes, margarine
Vitamin C	Ascorbic acid	Fresh fruits and vegetables
Vitamin E	Tocopherols	Vegetable oils, almond, maize, asparagus, tomatoes, peanuts
	Tocotrienols	Vegetable oils, wheat germ, barley, annatto, saw palmetto, nuts
Carotenoids	Lycopene	Autumn olive, tomatoes, watermelon, papaya
	Carotenes	Sweet potatoes, mustard greens, carrots, mangoes, broccoli, romaine lettuce, pumpkins
	Lutein	Spinach, kale, yellow carrots
Flavonoids	Quercetin (flavonols)	Citrus fruits and apples
	Genistein (isoflavones)	Soy bean, lupin, coffee
	Epigallocatechin-3-gallate (flavanols)	Tea
	Anthocyanin	Vaccinium species (blueberry), rubus berries (black berry), grape, black soybean

Table 1.2 continued

	Tannis	Tree bark, wood fruit, fruitpod, leaves and root
Phenolic acids	Caffeic acid	Coffee, tea
	Gallic acid	Land plants and aquatic plant (myriophyllum spicatum)
	Chlorogenic acid	Potato, bamboo, peach, prunes
Stillbenes	Resveratrol	Grape seeds and peel
Others	Curcumin	Turmeric

(Source: Combs, 2012; Pan et al., 2013; Horvath et al., 2006; Kim, 2014; Lemmens et al., 2014)

Numerous studies indicated that people with low intakes of antioxidant-rich fruits and vegetables were at greater risk for developing artery-clogging atherosclerosis, cancer or vision loss than the people who had large consumption of the fruits and vegetables. Clinical experiments have confirmed that these substances play important roles against heart disease, cancer, and other chronic diseases. The function and health benefit of some selected antioxidants are listed in Table 1.3 (Milbury et al., 2008).

Table 1.3 Function and health benefit of common antioxidants

Name	Function	Health Benefit
Vitamin A	Combining with protein opsin to form rhodopsin.	Inhibition of cancer, HIV, and dermatological disease.
Vitamin C	Rendering harmful free radical reactions harmless	Preventing or delaying food spoilage (enzymatic browning reaction)
Vitamin E	Breaking chains by preventing lipid oxidation	
Selenium	Defensing enzyme	Reducing the odds of prostate cancer
Carotenoids	Converting to vitamin A	Decreasing the risk of cancers and eye disease
Phytochemicals	Modulating cell metabolism and direct quenching of radicals	Increasing capillary strength and decreasing the risk of diseases

(Source: Milbury et al., 2008)

### 1.2.3 Antioxidant Activity

The antioxidant activity reflects the activity of antioxidants against the prooxidants or radicals (Apak et al., 2013). As the primary phytochemicals, phenolics have high antioxidant activity due to its phenoxyl radical could stabilize the whole structure by resonance after reacting with oxidants. It has been reported that the electronegative carboxyl or hydroxyl groups can increase the radical stability for the polyphenols. Also, carboxyl group could increase the antioxidant activity of the  $\beta$ -substituted monohydroxy acid (Rice-Evans et al., 1996). A typical proposed prooxidant reaction mechanism in lipid dispersions depicted with a galloyl group is demonstrated in Figure 1.3.

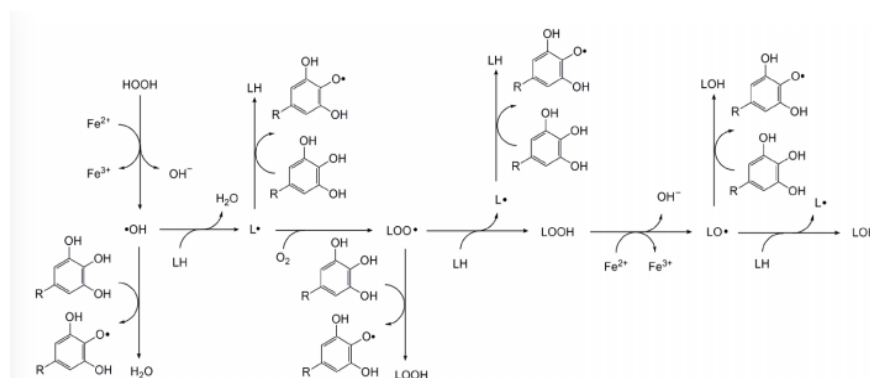


Figure 1.3 Mechanism in lipid dispersions. Abbreviations are as follows: hydroperoxyl radical (HOO•), hydroxyl radical (•OH), reduced lipid (LH), lipid alkyl radical (L•), lipid hydroperoxyl radical (LOO•), lipid hydroperoxide (LOOH), lipid alkoxy radical (LO•) (Zhou et al., 2012).

The methods to evaluate the antioxidant activity can be classified into two basic groups: electron transfer (ET) reaction – based redox titration and hydrogen atom transfer (HAT) reaction (Huang et al., 2005). The most popular ET-based assays are total phenolic assay, 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, cupric-reducing antioxidant capacity (CUPRAC), 2,2'-azino-bis (3-ethylbenzothiazoling-6-sulphonic acid) (ABTS) assay, ferric ion-reducing antioxidant power (FRAP) assay, and cerium(IV)-based reducing antioxidant capacity (CERAC) assay (Subramanian et al., 1965; Boguth et al., 1969; Apak et al., 2004; Miller et al., 1996; Benzie et al., 1996; Ozyurt et al., 2010). The



reaction of these assays is based on the mechanism that single electron oxidants react with reductant in samples to cause the color changes. These assays are very simple and commonly applied for screening the antioxidant activity of food and biological samples. On the other hand, oxygen radical absorbance capacity (ORAC) assay is a typical HAT-based assay (Cao et al., 1993).

For example, DPPH assay is developed by Brand Williams et al.(1995) as one of the oldest, simplest and most frequently used method to determine the antioxidant activity. When DPPH $\cdot$  reacts with an antioxidant, the color of DPPH $\cdot$  changes from purple to yellow (Figure 1.4) (Dorota Martysiak-Żurowska, 2012). Then, the change of the absorbance is measured by a spectrophotometer to calculate the antioxidant activity (Fadda et al., 2014).

CUPRAC assay is the result of antioxidants redox reaction with CUPRAC reagent and Cu(II)-neocuproine (Nc). The advantage of CUPRAC method is that it can be used for both lipophilic and hydrophilic antioxidants. Also, the pH of the assay is close to the physiological pH (Çelik, 2012; Çekic et al., 2012).

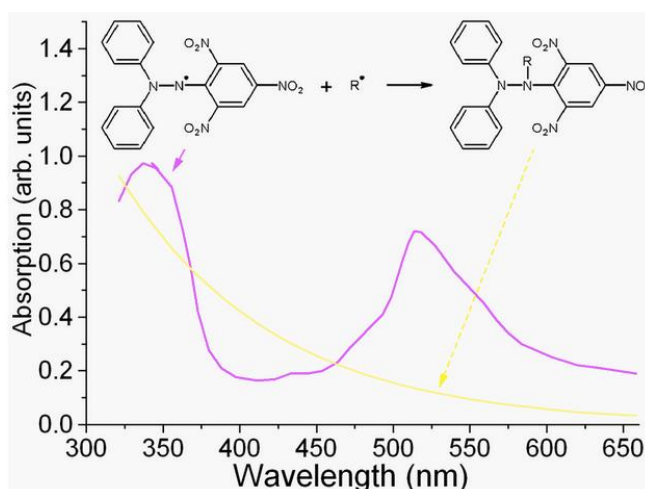


Figure 1.4 Absorbance wavelength of DPPH (Abuin et al., 2002)

ABTS assay is based on the oxidation of ABTS to generate colored radical cation -  $ABTS^{\cdot+}$  (blue/green color). The  $ABTS^{\cdot+}$  can be reduced by antioxidant in a wide pH range and applied for both lipophilic and hydrophilic antioxidants (Jia et al., 2012; Dawidowicz et al., 2013).

### 1.3 Potato and Sweet Potato

Potato (*Solanum tuberosum* L.) is the third largest production of food crop following rice and wheat in the world (Wang et al., 2015). Generally, potato is a tuber crop and began to be cultivated around 8000 years ago in the Central Andes region near Lake Titicaca (Peru-Bolivia), while, it started to be cultivated in 1719 in the United States. Belongs to the Solanaceae family, potato contains a large amount of carbohydrates and important protein, vitamins and minerals (Chung et al., 2014; Kunstelj et al., 2014). Sweet Potato (*Ipomoea batatas* L.) is another important crop widely cultivated in many Asian and African countries (Li, 2012). It is a stable perennial tuber crop and could be harvested after 90-120 days of cultivating (Kunstelj et al., 2014). There are a number of edible varieties of potatoes. The Andes of South America has the most diversity of potatoes in the world (Alyokhin et al., 2012). These potatoes can generally be divided into seven type categories: russet, red, white, yellow, blue/purple, fingerling and petite. Normally, the cooking method for potatoes is based on their texture. For example, russet potato contains low moisture and high starch content and is better for baking, mashing and frying (Zaidul et al., 2007). For sweet potatoes, their colors range from white to mild deep red. There are mainly eight varieties of sweet potatoes in America: Beauregard sweet potato (LA, 1987), Hernandez sweet potato (LA, 1992), Jewel sweet potato (NC, 1988), Carolina ruby sweet potato (NC, 1988), Porto Rico 198 sweet potato (NC, 1966), Cordner sweet potato

(TX, 1983), and White Delight sweet potato (GA). Jewel sweet potato, also called “the current Queen of sweet potatoes”, is the most common one in the markets.

### 1.3.1 Yields and Production for Potato and Sweet Potato

Potato is the third crop in the world because it is easy to be cultivated in the cool climate area. Developed countries produced more potatoes than developing countries from the year of 1950 to 2000 (Figure 1.5). Europe is used to be the biggest area to produce potatoes; however, Asia has expanded the areas to produce potato these years. Nowadays, China has the highest production of potato in the world and produces almost one third of potatoes together with Indian (Hijmans, 2001). According to a report from the Food and Agriculture Organization of the United Nations (FAO), over 365 million tons of potatoes were harvested in the world in 2012. For the US, the production of potato in 2013 was about 466 thousand tons. Base on the survey from United States Department of Agriculture (USDA), the US is the fourth potato production country in the world now. Most potatoes in the US are cultivated in the western states, mainly in Idaho and Washington State.

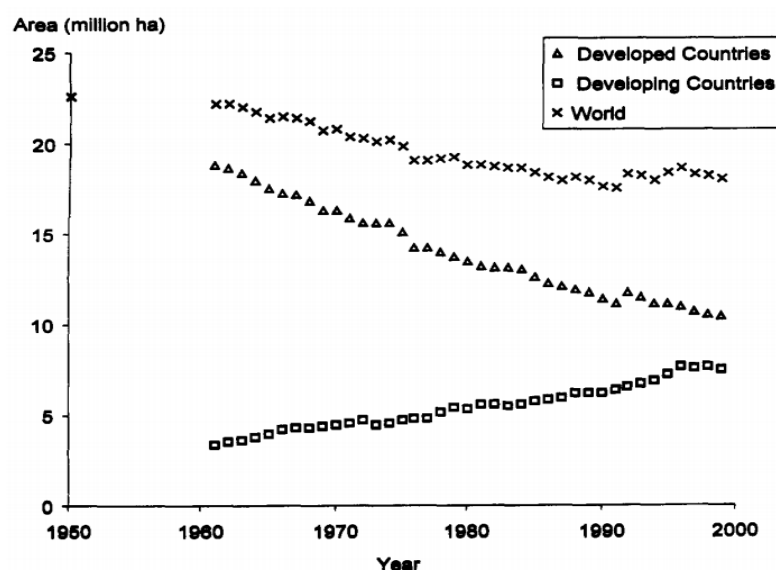


Figure 1.5 Global potato area over time: total and for developed and developing countries (Robert J. Hijmans, 2001)

Although sweet potato is adaptable to a wide difference of growing conditions, the best growing area is tropical, subtropical, and temperate areas (Hussein et al., 2014). Sweet potato is the top ten most consumed food staples in the world. According to the report from the United Nations FAO, over 106 million tons of sweet potatoes were harvested in the world in 2011. There are about nine million hectares for producing sweet potato in the world. Over 97% of sweet potatoes are produced from developing countries. China has 6.6 million hectares to grow sweet potato (Yan, 2014). China and Sub-Saharan Africa harvest almost 87% production of potatoes in the world (Raymundo et al., 2014). In the US, it is mainly produced in the southern states, especially Louisiana and North Carolina. The US production of sweet potato in 2013 was about 245 thousand tons.

### **1.3.2 Basic Nutrients and Toxics in Potato and Sweet Potato**

Potato is an economic and nutritive food source, providing a variety of health promoting ingredients (Alyokhin et al., 2012). These nutrients include vitamins, minerals, ascorbic acid,  $\alpha$ -tocopherol,  $\beta$ -carotene, and phenolic acids. It has been reported that potato is one of the most abundant sources of antioxidants in the human diet (Nems et al., 2015). The nutritional value for raw potato with skin is showed in Table 1.4. Sweet potato is a rich source of starches, complex carbohydrates,  $\beta$ -carotene and dietary fibers. In the areas such as Africa and India, sweet potato has significant effect on raising the vitamin A levels in human blood. Other nutrients including minerals phosphorus and calcium can also be found at a high level in sweet potatoes (Oladejo, 2013). Compared with other crops, the fat level in sweet potato is relatively low, while, the content of vitamin B, manganese and potassium in sweet potato is moderate (Kunstelj et al., 2014). The Center for Science in the Public Interest of USDA made comparison of the nutritional value (fiber, complex carbohydrates, protein, vitamin A and Potassium) of sweet potatoes and

other nine kinds of foods in 2001. The results revealed that the sweet potato is the healthiest food among them. The nutritional value for raw potato with or without skin is showed in Table 1.4.

Table 1.4 Nutritional values for potato and sweet potato (USDA Nutrient Database)

Nutritional value per 100g	Raw potato with skin	Raw sweet potato with skin	Cooked sweet potato in skin without salt
Energy	321 kJ (77 kcal)	359 kJ (90 kcal)	378 kJ (90 kcal)
Carbohydrates	17.47 g	20.1 g	20.7 g
Starch	15.44 g	12.7 g	7.05 g
Dietary Fiber	2.2 g	4.2 g	3.3 g
Sugar	NA	3 g	6.5 g
Fat	0.1 g	0.1 g	0.15 g
Protein	2 g	1.6 g	2.0 g
Vitamin A equiv.	NA	709 $\mu$ g (89%)	961 $\mu$ g (120%)
$\beta$ -catotene	NA	8509 $\mu$ g (79%)	NA
Thiamin (Vitamin B <sub>1</sub> )	0.08 mg (7%)	0.078 mg (7%)	0.11 mg (10%)
Riboflavin (Vitamin B <sub>2</sub> )	0.03 mg (3%)	0.061 mg (5%)	0.11 mg (9%)
Niacin (Vitamin B <sub>3</sub> )	1.05 mg (7%)	0.557 mg (4%)	1.5 mg (10%)
Pantothenic acid (Vitamin B <sub>5</sub> )	0.296 mg (6%)	0.8 mg (16%)	NA
Vitamin B <sub>6</sub>	0.295 mg (23%)	0.209 mg (16%)	0.29 mg (22%)
Folate (Vitamin B <sub>9</sub> )	16 $\mu$ g (4%)	11 $\mu$ g (3%)	6 $\mu$ g (2%)
Vitamin C	19.7 mg (24%)	2.4 mg (3%)	19.6 mg (24%)
Vitamin E	0.01 mg (0%)	0.26 mg (2%)	0.71 mg (5%)
Vitamin K	1.9 $\mu$ g (2%)	NA	NA
Calcium	12 mg (1%)	30 mg (3%)	38 mg (4%)
Iron	0.78 mg (6%)	0.61 mg (5%)	0.69 mg (5%)
Magnesium	23 mg (6%)	25 mg (7%)	27 mg (8%)
Manganese	0.153 mg (7%)	0.258 mg (12%)	0.5 mg (24%)

Table 1.4 continued

Phosphorus	57 mg (8%)	47 mg (7%)	54 mg (8%)
Potassium	NA	337 mg (7%)	475 mg (10%)
Sodium	6 mg (0%)	55 mg (4%)	36 mg (2%)
Zinc	0.29 mg (3%)	0.3 mg (3%)	0.32 mg (3%)
Water	75 g	NA	NA

(Source: Nutrient Data Laboratory, ARS, USDA, 2001)

The storage condition is very important for potatoes because improperly handling would speed up physiological process and loss the quality. Generally, potatoes should be carefully stored to keep them alive and slow the natural process of decomposition. For fresh-use, 7 °C is the preferred temperature, while, for long-term storage, it is better to store them at 4 °C. These two temperatures can minimize the production of glucose, an unwanted product reduced from sugar (Saour et al., 2012). If the storage temperature is not appropriate, starch in the potatoes could convert into sugars. This conversion will not only affect the taste of the potatoes, but also yield higher acrylamide levels in cooked potatoes. Besides acrylamide, glycoalkaloids such as solanine and chaconine are also potential toxic compound. The glycoalkaloids are synthesized in potatoes to protect them from predators. They are so stable in potatoes and can only be degraded over 170°C. When the potato's peel color turns green, it represents the production of toxic compound of solanine. Excessive consumption of these compounds may cause headaches, diarrhea, cramps or even death to human beings.

### 1.3.3 Food Application of Potato and Sweet Potato

Both potato and sweet potato can be consumed as fresh, processed to snack food, such as chips and fries. In the US, the consumption of potato is the second food staple following wheat (Hussein et al., 2014). They could be cooked as mashed baked, boiled or steamed

potatoes, as well as potato dumpling, pancakes, soup and salad etc. The cooking methods for sweet potatoes are similar to potatoes. Another popular way for potato and sweet potato food application is to produce french-fried potato and chip. French fries (“chips” in UK) are served world widely in restaurants for its fast reparation and special flavor (Tajner- Czopek et al., 2014). Chips are the most popular snack food in many developed countries, especially in the US. For example, about 21.6% of the potato production of the US was made into chips in 2001. In 1960, the consumption was 11.4 lbs. per person and increased to 19.3 lbs. in 2007. The consumption is increasing with years (Pedreschi et al., 2009). Also, dehydrated potato or sweet potato can be made into flour because they are rich in starch. Before extracting starch, the potato/sweet potato should be crushed to release starch granules. After washing and drying, the dried starch powder is ready to be used as disintegrants, glidants, and lubricants as they can bind the mucilaginous in pasta (Riley et al., 2010). Furthermore, they could be the important ingredients in making noodles, wine gums and cocktail nuts. In food industry, the starches are used as binder for meat mixtures and thicken gravy and soup (Zaidul et al., 2007).

#### **1.4 Phenolic Acids in Potato and Sweet Potato**

Phenolic compounds belong to the broader category of secondary metabolites in plants (Luthria et al., 2012). A variety of phenolic acids are found in different parts of plants such as the seeds, skins, leaves. If the stress condition of growing has changed, the role of phenolics in the metabolic system will change as well (William et al., 1979). For instance, cinnamic acid and its derivatives are the major phenolic compounds in corn, cereal grains, red wine and citrus fruits. Caffeic, *p*-coumaric, ferulic, and sinapic acids are abundant in the mentioned foods (Devanand L. Luthria et al., 2012). Phenolic acids are also found in honey and can be used to determine the floral origin (Eliana Spilioti et al., 2014). Tea and

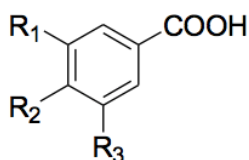
grape seeds are rich in gallic acid, while caffeic and chlorogenic acids are the main phenolics in coffee. Fruits such as kiwi, berries and apples contain high content of caffeic acid. Among the root plants, potato and sweet potato have relatively high varieties of phenolics, which are commonly recognized as antioxidants (Lemmens et al., 2014).

In potato, over 50% of the total phenolic acids are caffeic acid, cinnamic acid, p-coumaric acid, ferulic acid, sinapic acid, and chlorogenic acid (Nems et al., 2015). As the major phytochemical in potatoes, chlorogenic acid has different isomeric forms, such as 3-O-caffeoylquinic (n-chlorogenic acid), 4-caffeoylquinic (crypro-chlorogenic acid), and 5-O-caffeoylquinic (neo-chlorogenic acid) (Lthria et al., 2012). Sweet potato is another chlorogenic acid rich source (Rudkin and Nelson, 1947). The phenolic acids in sweet potato mainly consist of chlorogenic acid and other similar compounds (Nandutu et al., 2007). A previous study showed that six types of phenolic acids have been found in sweet potato: neochlorogenic acid (3-CQA), cryptochlorogenic acid (4-CQA), chlorogenic acid (5-CQA), and three isochlorogenic acid isomers—isochlorogenic acid A (3,5-diCQA), isochlorogenic acid B (3,4-diCQA) and isochlorogenic acid C (4,5-diCQA) (Finotti et al., 2012). These phenolics, related to color and flavor of the plant, can protect the damage caused by pests (Lin et al., 2007). Also, they are important factors to the texture and nutrition value of vegetable foods because they can be a part of lignin and dietary fiber. It was found that the total phenolic content or antioxidant capacity of colored sweet potatoes was correlated with the total anthocyanins content (Vreugdenhill et al., 2007).

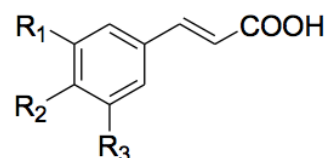


### 1.4.1 Chemical Structures of Phenolic Acids

Phenolic compounds in plant could generally be divided into three groups: phenolic acids, flavonoids, and tannins. Phenolic acid has one or more aromatic rings with multiple hydroxyl groups in either free or bound form in plants (Dai, 2010; Mattila et al, 2007). There are two forms of phenolic acids in potato - soluble and insoluble bound forms. The soluble form is the free and soluble esters or soluble glycosides. Most of phenolic acids are the derivatives from the hydroxycinnamic acid (free form) and hydroxybenzoic acid (bound form) (Shahidi et al., 1995). The main difference between the structures of these two categories is the patterns of the aromatic rings of their derivatives (Figure 1.6) (Lall et al., 2015). Chlorogenic acid, caffeic acid and ferulic acid are hydroxycinnamic acid in potato while gallic acid and protocatechuic acid as well as their derivatives are the common hydroxybenzoic acids in potato (Albishi, 2013). The structures of the phenolic acids are showed in Figure 1.7.



Hydroxybenzoic acid



Hydroxycinnamic acid

Figure 1.6 Structures of hydroxybenzoic acid and hydroxycinnamic acid.  $R_{1,2,3}$  can be OH, H, or  $\text{CH}_3\text{O}$  (Lall et al., 2015).

There are three main isomers for chlorogenic acids: 3-O-caffeoylquinic (n-chlorogenic acid), 4-O-caffeoylquinic (crypro-chlorogenic acid) and 5-O-caffeoylquinic (neo-chlorogenic acid). 3,4-di-O-caffeoylquinic acid, 3,5-di-O-caffeoylquinic acid and 4,5-di-O-caffeoylquinic acid were also common in potato and sweet potato (Table 1.5) (Jaiswal et al., 2010).

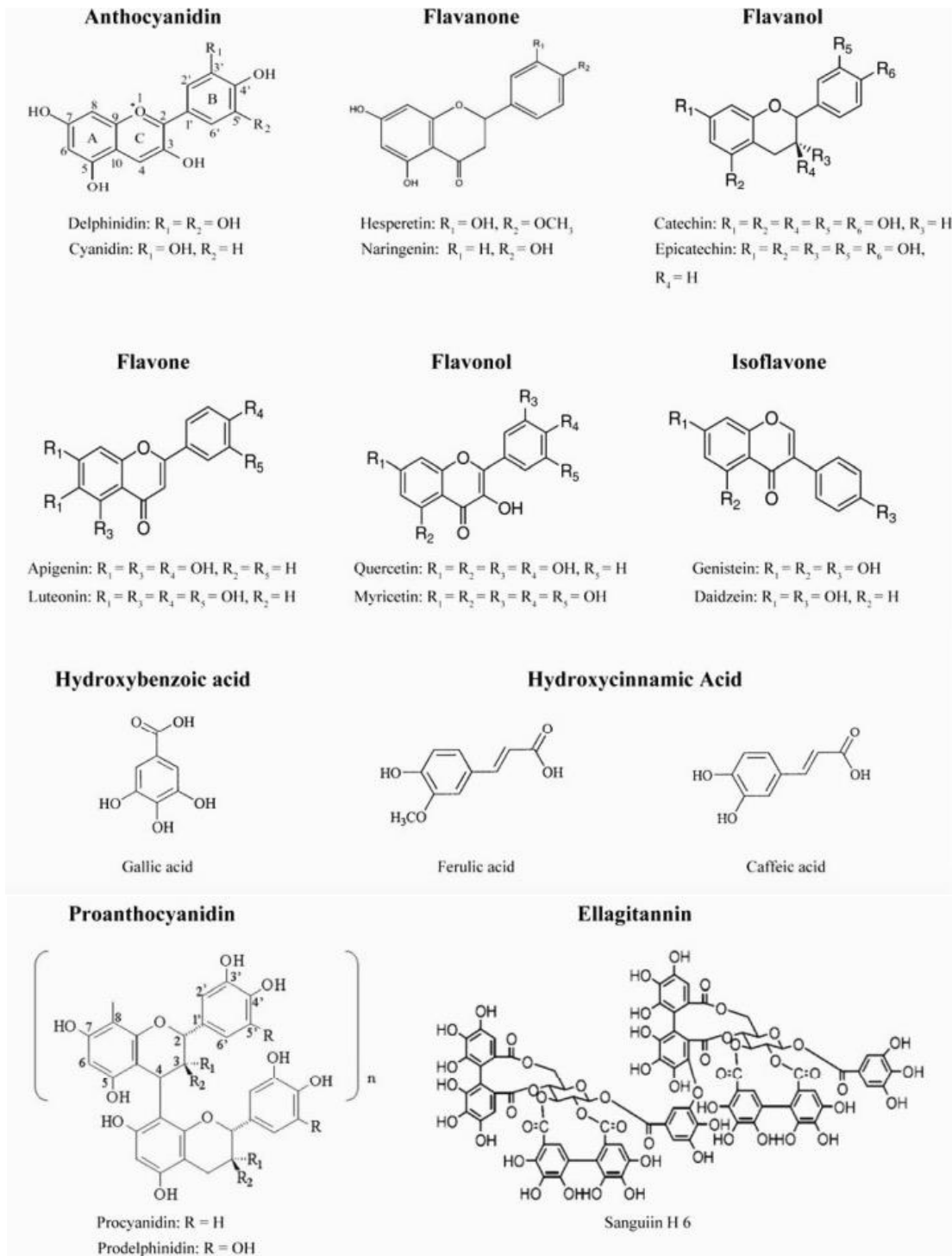
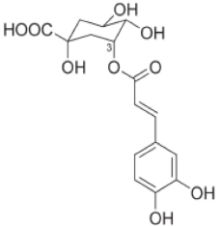
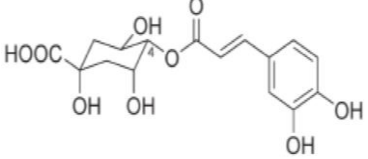
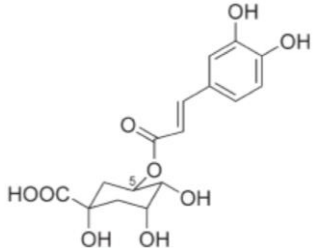
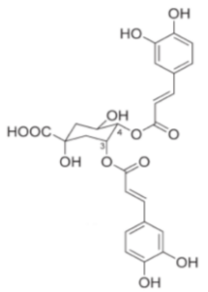
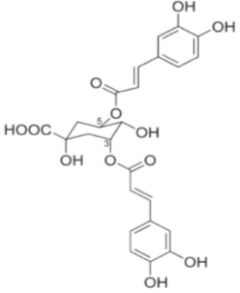
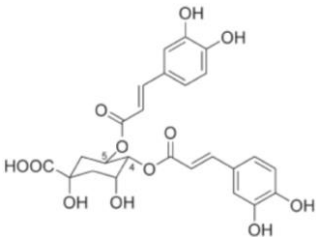


Figure 1.7 Structures of flavonoids, phenolic acids and tannins (Dai, 2010).

Table 1.5 Structures of Chlorogenic Acids

Name	Abbreviation	Structure	R
3-O-caffeoylquinic	3-CQA		$R^3=C$ $R^4=H$ $R^5=H$
4-O-caffeoylquinic	4-CQA		$R^3=H$ $R^4=C$ $R^5=H$
5-O-caffeoylquinic	5-CQA		$R^3=H$ $R^4=H$ $R^5=C$
3,4-di-O-caffeoylquinic acid	3,4-diCQA		$R^3=C$ $R^4=C$ $R^5=H$
3,5-di-O-caffeoylquinic acid	3,5-diCQA		$R^3=C$ $R^4=H$ $R^5=C$
4,5-di-O-caffeoylquinic acid	4,5-diCQA		$R^3=H$ $R^4=C$ $R^5=C$

(Source: Jaiswal et al., 2010)

### 1.4.2 Health Benefits of Phenolic Acids for Human

Since phenolic acids could exhibit antibacterial, antiviral, anticarcinogenic, anti-inflammatory and vasodilatory performance, they have been used for protection of food and cells from oxidative degeneration (Mattila et al., 2007; Nandutu et al., 2007). Also, phenolic acids are reported to have the ability to inhibit the growth of human cancer cells and decrease the possibility of hepatoma and human immunodeficiency virus (HIV) replication (Zhu et al., 1999; Yagasaki et al., 2000; Finotti et al., 2012). Chlorogenic acid was reported to inhibit the growth of an angiogenic enzyme (matrix metalloproteinase-9), which is associated with tumor invasion and metastasis. Caffeoylquinic acids can work as antimutagen in the Ames *Salmonella* assay (Jung et al., 2011). In fact, phenolic compounds could affect many cellular processes and possess a variety of biochemical features and functions. The multiple cellular responses and processes of inhibiting the growth of cancer cells are shown in Figure 1.8.

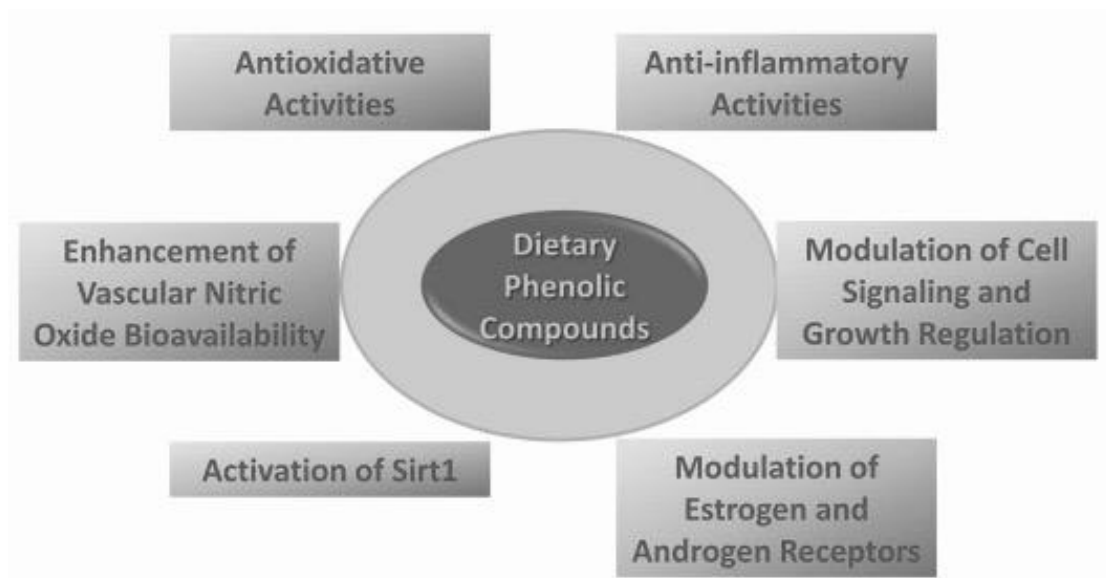


Figure 1.8 Dietary phenolic compounds as multitasking molecules (Li, 2011).

The objective for this research is to compare the concentration of phenolic acids in different raw and boiled potatoes and sweet potatoes, and determine the best potato/sweet potato that contains the highest concentration of phenolic acids. Secondly, the antioxidant activity and concentration of phenolic acids at different boiling times will be analyzed in the potato/sweet potato to show the highest concentration of phenolic acids. In addition, our goal was to provide the reference of the best time for potatoes/sweet potatoes that could yield the highest antioxidants content during boiling.

## CHAPTER 2. PHENOLIC ACIDS IN RAW AND BOILED POTATOES AND SWEET POTATOES

### 2.1 Introduction

Polyphenols are a class of natural compounds that have numerous hydroxyl groups attached to aromatic rings (Lall et al., 2015). Among polyphenols, phenolic acids have a potent antioxidant activity by reacting active oxygen and nitrogen species (Plazas et al., 2014). Generally, phenolic acids can be divided into two classes – hydroxybenzoic and hydroxycinnamic acids. Compared with phenylacetic, phenyl-lactic, phenylmandelic and phenyl-hydracrylic acids, other types such as phenylvaleric and phenylpropionic acids are more common in plants (Indu and Alan, 2010). The most well-known hydroxycinnamic acid is chlorogenic acid which could combine with another two common hydroxycinnamic acids – caffeic and quinic acids. On the other hand, the common form of hydroxybenzoic acids in food is p - hydroxybenzoic, vanillic and protocatechuic acids (Mattila et al., 2007).

As the intake of phenolic antioxidants has been confirmed to have health benefit in preventing chronic disease, the Food and Drug Administration (FDA) estimates that there are over 29,000 dietary antioxidant supplements sold in market with about 1,000 new products developed annually (Milbury et al., 2008). Considered as the common, cheap, and healthy food source, potato and sweet potato contain high amount of antioxidants, especially phenolic acids. In potato, about 80% of phenolic acids are chlorogenic acid and its related derivatives. Chlorogenic acid is the esterification product of caffeic acid and quinic acid which has been associated with health promoting functions. Some clinical studies have reported that chlorogenic acid could exhibit analgesic, anti-carcinogenic, anti-diabetic, anti-inflammatory, anti-microbial, anti-obesity, cardioprotective,

hypotensive and neuroprotective effects (Plazas et al., 2014). The content of phenolic compounds in potato and sweet potato is depended on the varieties (flesh color). Potato and sweet potato with red and blue flesh color contain more phenolic compounds than yellow and white flesh color potato (Rytel et al., 2014). The level of phenolic acids in sweet potato with red flesh was higher than in blueberry. Blueberry is a kind of fruit with high amount of antioxidants (Cevallos-Casals and Cisneros-Zevallos, 2003).

The phenolic compounds were usually extracted with organic solvents from plant material, such as methanol, acetone, ethanol and ethyl acetate (Dai and Murpher, 2010; Svennsson et al., 2010). In plant extract, phenolics quantification is effected by the chemical structure of phenolics, extraction methods, and possible interfering substances (Naczk and Shahidi, 2006). The method to quantify classes of phenolics in plant was the traditional spectrophotometric assays. Gas chromatographic (GC) techniques were widely used in separating and quantifying phenolic acids. However, the volatility of phenolics was low and should be transferred into the derivatives with high volatility (Stalikas, 2007). Compared with GC techniques, high performance liquid chromatographic (HPLC) techniques were more popular and reliable to analyze phenolics (Prior et al., 2001). They can analyze all the interests with their derivatives or degradation products simultaneously (Sakakibara et al., 2003; Downey and Rochfort, 2008). It was reported that reversed-phase columns could enhance HPLC separation of phenolics and C18 were the best column (Oh et al., 2008). For the mobile phase, acetonitrile and methanol were the widely used organic modifiers; acetic, formic or phosphoric acid was the commonly used acidified modifier, because they can minimize the peak tailing (Stalikas, 2007; Naczk and Shahidi, 2004; Robbins, 2003; Merken and Beecher, 2000).

The objective of the research in this part were two folds: 1) to determine the concentration of phenolic acids in different raw and boiled potatoes and sweet potatoes, 2) to find the sample with highest total concentration of phenolic acids and most various varieties of phenolic acids. As antioxidant activity is related to the level of phenolic acids, the sample with higher content of phenolic acids may have higher antioxidant activity.

## 2.2 Material and Methods

### 2.2.1 Sample Collection

Three types of potatoes – Loose Russet Potato (RP), Loose Gold Potato (GP), Loose Red Potato (ReP), and four types of sweet potatoes – Red Garnet Sweet Potato (RGSP), Hannah Sweet Potato (HSP), Jewel Sweet Potato (JeSP), Japanese Sweet Potato (JaSP) were bought from the “Whole Foods Market” at Baton Rouge, LA (Figure 2.1). These potatoes and sweet potatoes were harvested in the US.

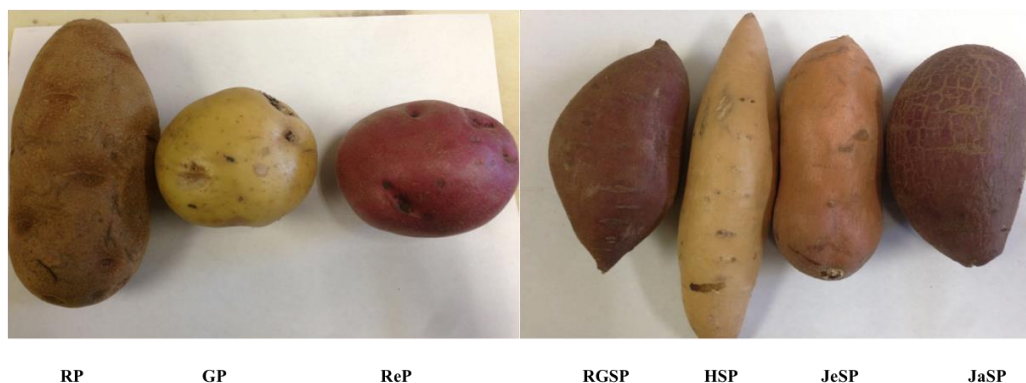


Figure 2.1 Potato and sweet potato categories

### 2.2.2 Sample Preparation

Raw potatoes and sweet potatoes were peeled, cut into pieces ( $2 \times 2 \times 2$  cm) and blended by a kitchen blender. Then, the mash was collected and stored in a 4 °C refrigerator before



use. The boiled samples were boiled in 100 °C water for 20 min before peeled. Their mashes were collected and stored in a 4 °C refrigerator before use.

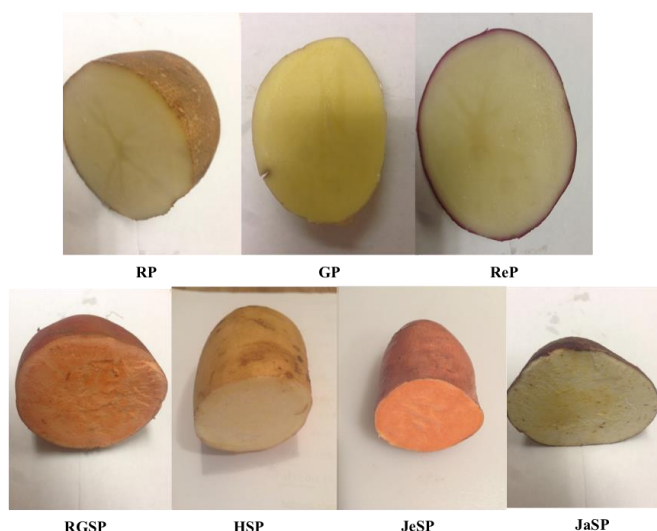


Figure 2.2 Pictures for the flesh of potato and sweet potato samples

### 2.2.3 Moisture Analysis for Raw and Boiled Samples

The method – loss on drying (LOD) – was used in this study to determine moisture content in the samples. Fourteen foil dishes were weighed and labeled. Five grams of the raw or boiled sample was placed on the labeled foil and recorded. After dried at 80°C in an oven for six hours, the samples were transferred to a desiccator to cool down before reweighed.

### 2.2.4 Extraction of Phenolic Acids for Potato and Sweet Potato Samples

The samples (1.0 g) were mixed with methanol (2 mL) in a 10 mL tube. The mixture was mixed thoroughly by vortex for 1 min and then incubated in a water bath at 60°C. After 20 min, the mixture was centrifuged. The solvent layer was transferred to a clean test tube. The residue was extracted with another 2 mL of methanol. The solvent layers were combined and concentrated for HPLC analysis.

## 2.2.5 Determination of Phenolic Acids by Using an HPLC Method

High performance liquid chromatography (HPLC, Water 2690 Separation Module) was equipped with diode array detector (DAD) which was set to scan from 210 to 620 nm. The identification and quantification of phenolic acids were carried out by using a C<sub>18</sub> Column (250 × 4.6 mm). The wavelength for the identification was set at 280 nm for gallic acid and 326 nm for chlorogenic acids and its derivatives. The extract solution (20 μL) from each potato or sweet potato sample was injected into the HPLC system. The mobile phase consisted of 10% acetic acid (A) and acetonitrile (B). A gradient elution program was employed as 100% A, 0 min; 70% A, 50 min; 50% A, 70 min; 20% A, 80 min; 0% A, 85 min; 100% A, 90min and hold 20 min. The flow rate set at 0.8 mL/min.

The results were analyzed by ANOVA (SAS) to observe the difference between the data collected at different conditions.

## 2.3 Results and Discussion

### 2.3.1 Moisture Content

In this study, the moisture of RGSP sample (81.9%) was the highest among the raw samples. The highest moisture content among the boiled samples was in ReP sample (81.5%). For both raw and boiled samples, the moisture content of JaSP was the lowest one (Table 2.1). The results were in agreement with the study of Chung (2014) which reported that the moisture content of potatoes was around 80%.

Table 2.1 Moisture of raw and boiled potato and sweet potato samples

Sample Type	RP	GP	ReP	RGSP	HSP	JeSP	JaSP
Raw	79.9	78.4	81.2	81.9	74.8	79.7	67.8
Boiled	81.5	75.0	83.0	80.1	77.6	77.2	73.0

RP: loose russet potato; GP: loose gold potato; ReP: loose red potato; RGSP: red garnet sweet potato; HSP: hannah sweet potato; JeSP: jewel sweet potato; JaSP: Japanese sweet potato.

### 2.3.2 Phenolic Acids in Potato Samples

Seven types of phenolic acids gallic acid, 3-CQA (neochlorogenic acid), 5-CQA (chlorogenic acid), 4-CQA (cryptochlorogenic acid), 4,5-diCQA (isochlorogenic acid), 3,5-diCQA (isochlorogenic acid), and 3,4-diCQA (isochlorogenic acid) were determined in this study (Figure 2.3-2.9). The diversities and initial concentration of phenolic acids in the fresh potato samples were relatively low, compared with the sweet potato samples. After boiled, the level of gallic acid in RP was not changed, while 3-CQA in GP, ReP, RGSP, HSP, JeSP, and JaSP as well as the 3,4-diCQA in RGSP decreased. However, other phenolic acids increased in all the potato samples (Figure 2.3-2.9). In addition, after boiled, there were two new phenolic acids (N1 and N2) in potato samples and one new phenolic acid (N3) in sweet potato samples (Figure 2.10-2.12). Gallic acid was the most common phenolic acid in the potatoes, while 3,4-diCQA was only in sweet potatoes (Figure 2.3 and 2.9).

There were five kinds of phenolic acids found in potato samples: gallic acid, 3-CQA, 5-CQA, 4-CQA, and 4,5-diCQA (Figure 2.3-2.7). Also, two kinds of new phenolic acids N1 and N2 were detected in boiled potato samples (Figure 2.10 and 2.11). However, 3,5-diCQA and 3,4-diCQA were not detected in both fresh and cooked potato samples (Figure 2.8 and 2.9).

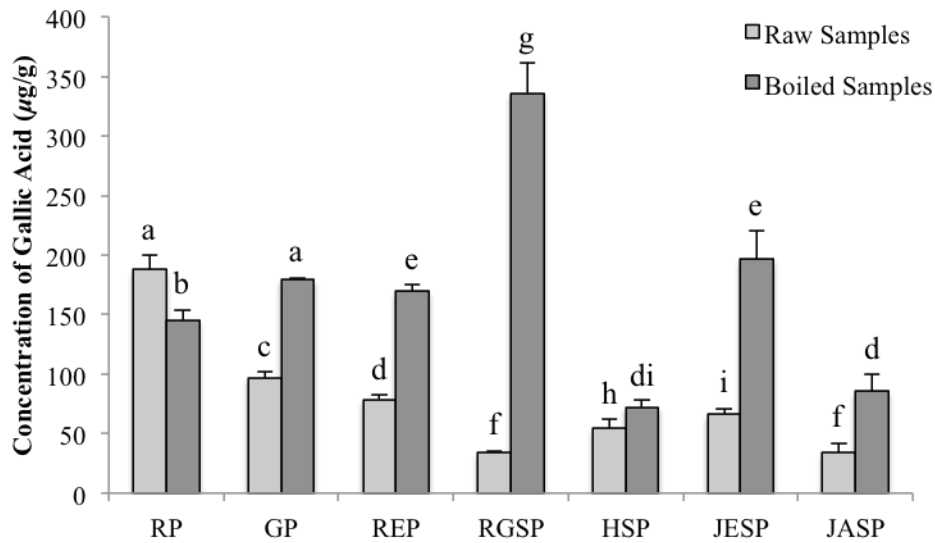


Figure 2.3 Concentration of gallic acid in potato and sweet potato samples  
 RP: loose russet potato; GP: loose gold potato; ReP: loose red potato; RGSP: red garnet sweet potato; HSP: hannah sweet potato; JeSP: jewel sweet potato; JaSP: Japanese sweet potato. Means with different letters are significantly different at  $P < 0.05$ .

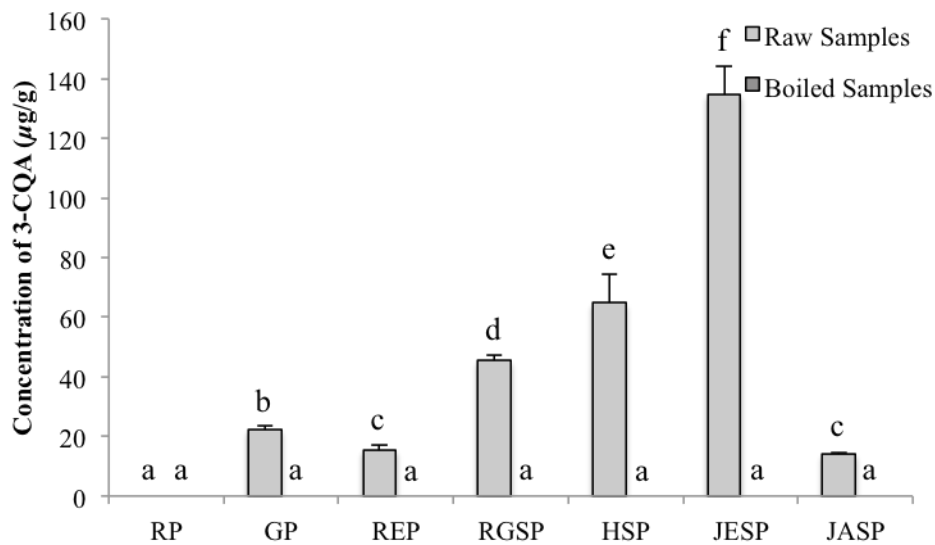


Figure 2.4 Concentration of 3-CQA in potato and sweet potato samples  
 RP: loose russet potato; GP: loose gold potato; ReP: loose red potato; RGSP: red garnet sweet potato; HSP: hannah sweet potato; JeSP: jewel sweet potato; JaSP: Japanese sweet potato. Means with different letters are significantly different at  $P < 0.05$

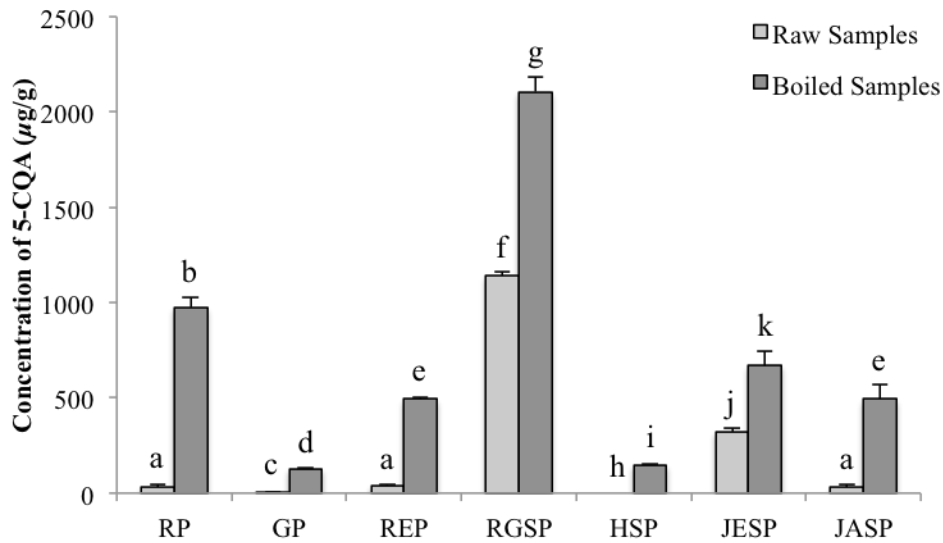


Figure 2.5 Concentration of 5-CQA in potato and sweet potato samples  
 RP: loose russet potato; GP: loose gold potato; ReP: loose red potato; RGSP: red garnet sweet potato; HSP: hannah sweet potato; JeSP: jewel sweet potato; JaSP: Japanese sweet potato. Means with different letters are significantly different at  $P < 0.05$

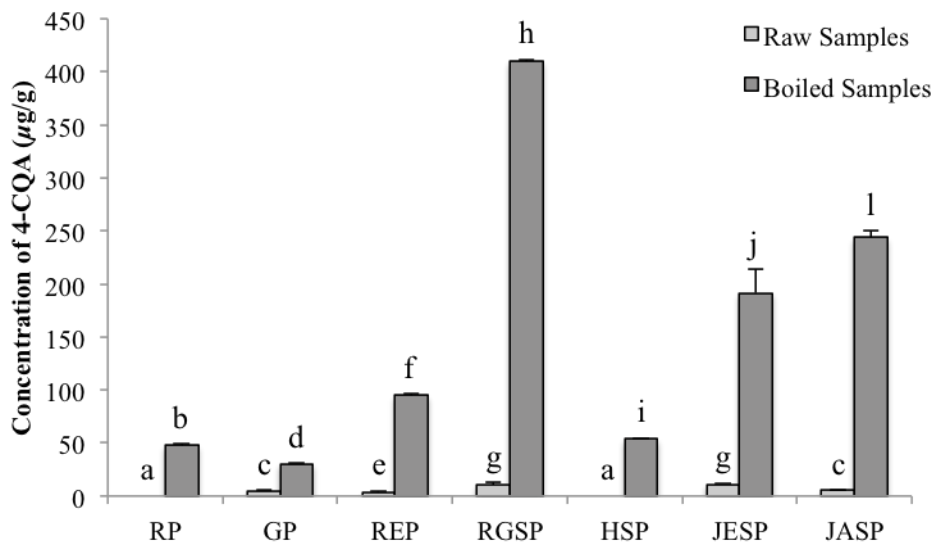


Figure 2.6 Concentration of 4-CQA in potato and sweet potato samples  
 RP: loose russet potato; GP: loose gold potato; ReP: loose red potato; RGSP: red garnet sweet potato; HSP: hannah sweet potato; JeSP: jewel sweet potato; JaSP: Japanese sweet potato. Means with different letters are significantly different at  $P < 0.05$ .

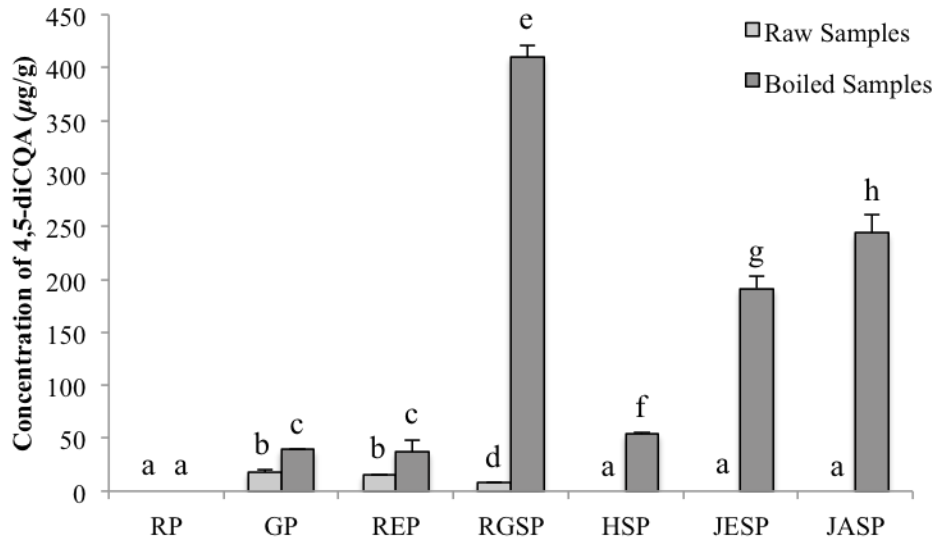


Figure 2.7 Concentration of 4,5-diCQA in potato and sweet potato samples  
 RP: loose russet potato; GP: loose gold potato; ReP: loose red potato; RGSP: red garnet sweet potato; HSP: hannah sweet potato; JeSP: jewel sweet potato; JaSP: Japanese sweet potato. Means with different letters are significantly different at  $P < 0.05$ .

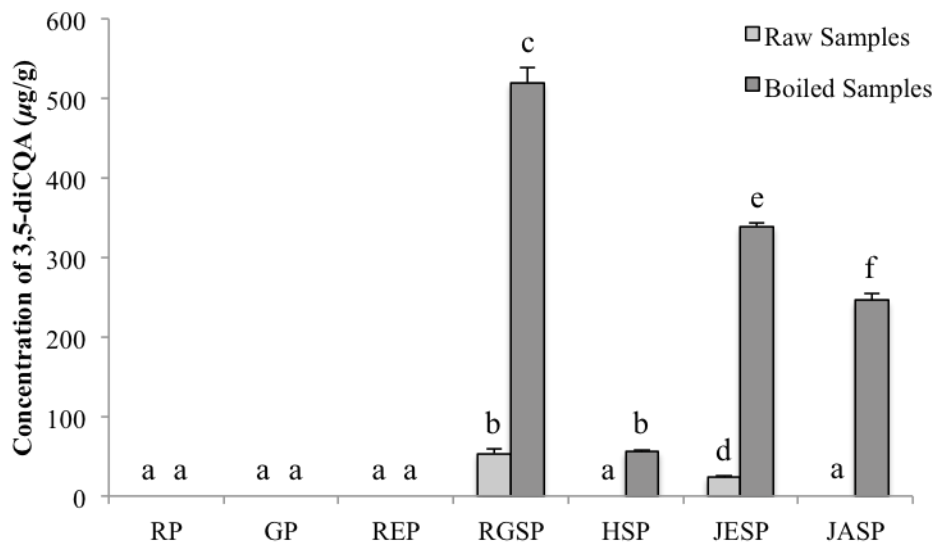


Figure 2.8 Concentration of 3,5-diCQA in potato and sweet potato samples  
 RP: loose russet potato; GP: loose gold potato; ReP: loose red potato; RGSP: red garnet sweet potato; HSP: hannah sweet potato; JeSP: jewel sweet potato; JaSP: Japanese sweet potato. Means with different letters are significantly different at  $P < 0.05$ .

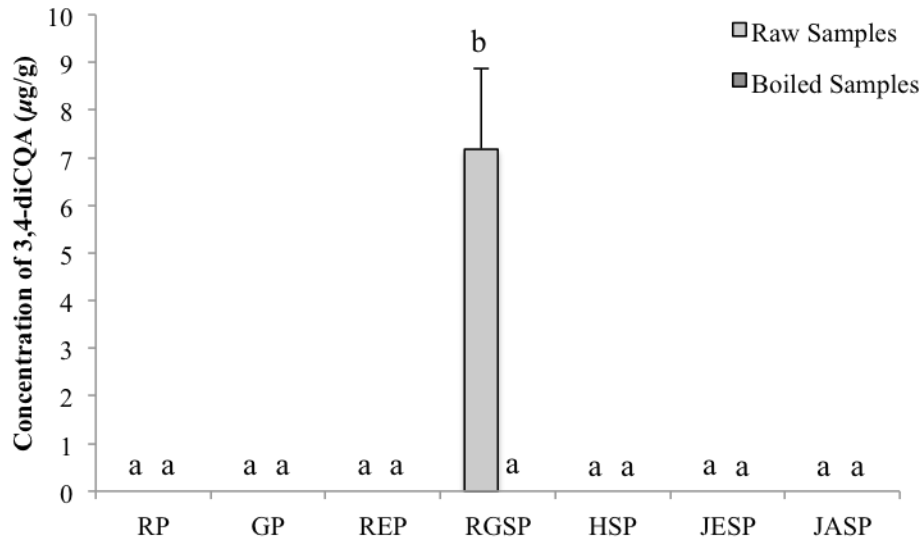


Figure 2.9 Concentration of 3,4-diCQA in potato and sweet potato samples  
 RP: loose russet potato; GP: loose gold potato; ReP: loose red potato; RGSP: red garnet sweet potato; HSP: hannah sweet potato; JeSP: jewel sweet potato; JaSP: Japanese sweet potato. Means with different letters are significantly different at  $P < 0.05$ .

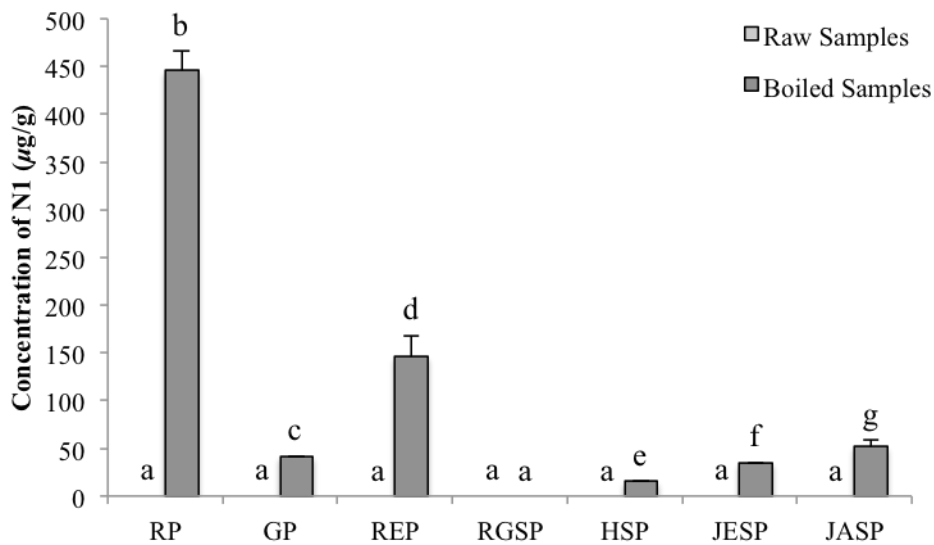


Figure 2.10 Concentration of new phenolic acid (N1) in potato and sweet potato samples.  
 RP: loose russet potato; GP: loose gold potato; ReP: loose red potato; RGSP: red garnet sweet potato; HSP: hannah sweet potato; JeSP: jewel sweet potato; JaSP: Japanese sweet potato. Means with different letters are significantly different at  $P < 0.05$ .

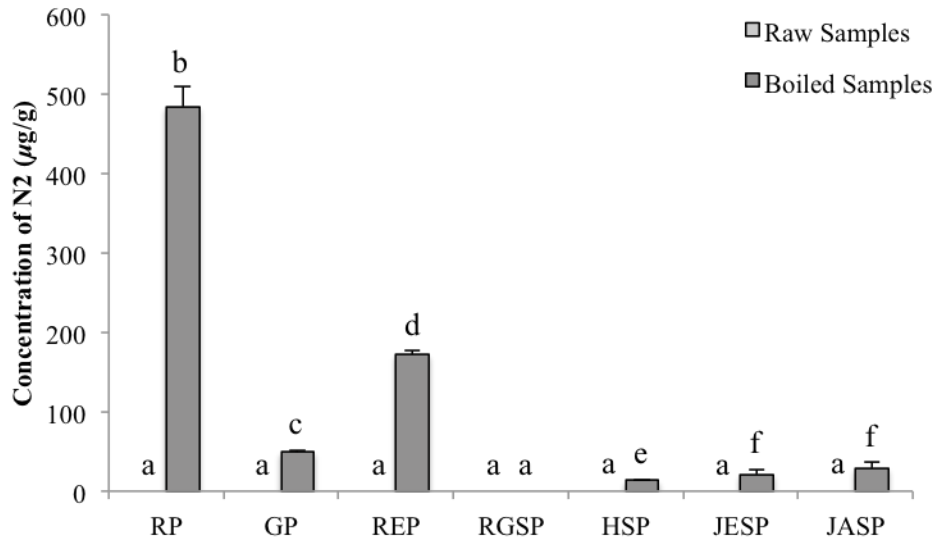


Figure 2.11 Concentration of new phenolic acid (N2) in potato and sweet potato samples. RP: loose russet potato; GP: loose gold potato; ReP: loose red potato; RGSP: red garnet sweet potato; HSP: hannah sweet potato; JeSP: jewel sweet potato; JaSP: Japanese sweet potato. Means with different letters are significantly different at  $P < 0.05$ .

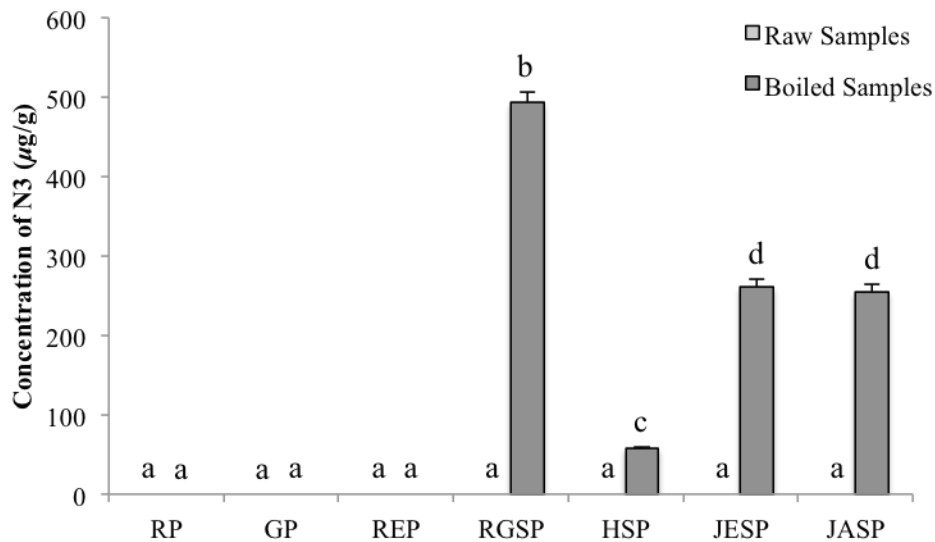


Figure 2.12 Concentration of new phenolic acid (N3) in potato and sweet potato samples. RP: loose russet potato; GP: loose gold potato; ReP: loose red potato; RGSP: red garnet sweet potato; HSP: hannah sweet potato; JeSP: jewel sweet potato; JaSP: Japanese sweet potato. Means with different letters are significantly different at  $P < 0.05$ .



In raw RP sample, only two phenolic acids gallic acids and 5-CQA were detected, while gallic acid, 3-CQA, 5-CQA, 4-CQA, and 4,5-diCQA were found in raw GP and ReP samples. The concentrations and profiles of phenolic acids in the potato samples were summarized in Table 2.2. Gallic acid was the highest concentration phenolic acid in all raw samples. Nevertheless, the concentration of 4-CQA was the lowest among the raw samples and approximately 60 times lower than that of gallic acid. The overall phenolics concentration of RP was the highest while it was the lowest in GP sample.

For the potato with high phenolic acid concentration, it has high potential of browning because polyphenol oxidase in the plants would be released to catalyze the phenolics into brown colored quinones (Wang et al., 2015). By exposing to air for the same period of time, the flesh of RP was the easiest sample to taking place of browning. It was in agreement with the suggestion of that the high concentration of phenolic acids results in the fastest rate of browning reaction.

Table 2.2 Concentration of new phenolic acids in potato samples

Concentration ( $\mu\text{g/g}$ )		N1	N2
Raw	RP	ND	ND
	GP	ND	ND
	ReP	ND	ND
Boiled	RP	445.8 $\pm$ 20.9 <sup>a</sup>	484.4 $\pm$ 24.7 <sup>a</sup>
	GP	41.5 $\pm$ 0.6 <sup>b</sup>	49.7 $\pm$ 2.0 <sup>b</sup>
	ReP	146.3 $\pm$ 22.2 <sup>c</sup>	172.6 $\pm$ 4.6 <sup>c</sup>

N1, N2: New compounds after boiling; ND: not detected. RP: loose russet potato; GP: loose gold potato; ReP: loose red potato; Means with different letters are significantly different at  $P < 0.05$ .

Table 2.3 Concentration of phenolic acids in potato samples

Concentration ( $\mu\text{g/g}$ )		Gallic Acid	P1	P2	P3	P4
Raw	RP	187.8 $\pm$ 12.5 <sup>a</sup>	ND	32.6 $\pm$ 14.6 <sup>a</sup>	ND	ND
	GP	96.8 $\pm$ 5.0 <sup>b</sup>	22.3 $\pm$ 1.1 <sup>a</sup>	5.6 $\pm$ 2.6 <sup>b</sup>	4.9 $\pm$ 0.5 <sup>a</sup>	18.0 $\pm$ 2.8 <sup>a</sup>
	ReP	78.9 $\pm$ 4.0 <sup>c</sup>	15.4 $\pm$ 1.6 <sup>b</sup>	39.2 $\pm$ 5.0 <sup>a</sup>	3.2 $\pm$ 0.9 <sup>b</sup>	15.0 $\pm$ 0.2 <sup>b</sup>
Cooked	RP	145.4 $\pm$ 8.2 <sup>d</sup>	ND	975.4 $\pm$ 51.4 <sup>c</sup>	47.8 $\pm$ 1.2 <sup>c</sup>	ND
	GP	179.7 $\pm$ 0.4 <sup>a</sup>	ND	128.0 $\pm$ 5.4 <sup>d</sup>	30.4 $\pm$ 0.9 <sup>d</sup>	39.1 $\pm$ 0.3 <sup>c</sup>
	ReP	170.0 $\pm$ 5.2 <sup>e</sup>	ND	493.7 $\pm$ 9.7 <sup>e</sup>	94.8 $\pm$ 1.6 <sup>e</sup>	37.1 $\pm$ 11.5 <sup>c</sup>

P1: 3-CQA (neochlorogenic acid); P2: 5-CQA (chlorogenic acid); P3: 4-CQA (cryptochlorogenic acid); P4: 4,5-diCQA (isochlorogenic acid); ND: not detected. RP: loose russet potato; GP: loose gold potato; ReP: loose red potato; Means with different letters are significantly different at  $P < 0.05$ .

After boiling, 4-CQA was detected in cooked RP sample while 23% of gallic acid was degraded. However, 5-CQA increased by 28 times (Table 2.3). In GP and ReP samples, 3-CQA was degraded after boiling, while gallic acid increased 85% and 115%, respectively. Also, 5-CQA, 4-CQA and 4,5-diCQA in GP increased approximately 22, 5, and 117%, respectively. The increase rates were 12, 29 times and 147% for 5-CQA, 4-CQA and 4,5-diCQA in ReP sample, respectively (Table 2.3). It was reported that concentration of gallic acid increased in red wine after aging due to the breaking down of galloylated procyanidins and hydrolysis of glycoside of gallic acid (Alcalde-Eon et al., 2014). It may have similar reaction for the increasing of gallic acid in the potato samples after boiling. In addition, a previous study showed that chlorogenic acid was not stable under high temperature (range of 125 °C to 225°C) (Zanoelo et al., 2009). Also, the isomer of caffeoylquinic acids may transfer into 4-CQA and 5-CQA during boiling.

Two new compounds N1 and N2 were detected in the boiled potatoes. They have the same maximum absorbance wavelength in their spectrums. The concentration of N1 or N2 in RP was the highest among the samples. However, GP had the lowest N1 and N2 concentrations (Table 2.2). According to the study of Antolovich et al. in 2000, caffeic acid was produced in boiled potato. N1 and N2 had similar spectrum as caffeic acid and may be caffeic acid derivatives. The occurrence of caffeic acid was due to caffeoylquinic acid can be hydrolyzed to caffeic acid, and the most possible caffeoylquinic acid could be 3-CQA (Figure 2.14) (Antolovich et al, 200).

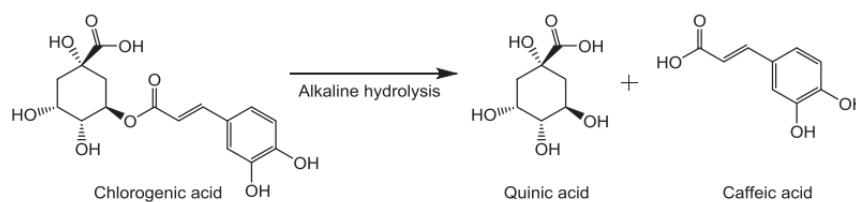


Figure 2.14 Products of alkaline hydrolysis of chlorogenic acid (Maldonado et al., 2014)

### 2.3.3 Phenolic Acids in Sweet Potato Samples

Seven phenolic acids including gallic acid, 3-CQA, 5-CQA, 4-CQA, 4,5-diCQA, 3,5-diCQA and 3,4-diCQA were detected in the sweet potato samples (Figure 2.3-2.9). Also, three types of new phenolic acids were found in the boiled sweet potato samples and expressed as N1, N2 and N3 (Figure 2.10-2.12). Among the raw sweet potato samples, RGSP sample was the only one that had all the seven phenolic acids with the highest concentration as well. For JeSP and JaSP samples, gallic acid, 3-CQA, 5-CQA, and 4-CQA were determined while only gallic acid and 3-CQA were detected in HSP. Previous research showed that in most sweet potatoes, the predominant phenolic acids were 5-CQA and 3,5-diCQA (Padda and Picha, 2008c).

The total phenolic acid level is usually correlated with the color of the potato flesh. For example, the sweet potato with purple and red color flesh may have more diversities and higher level of phenolic acids than yellow and white flesh sweet potatoes (Ji et al., 2012). Moreover, the sweet potato with white flesh contains more chlorogenic acid, while the sweet potato with purple flesh contains more di-CQAs (Cevallos-Casals and Cisneros-Zevallos, 2003). After boiling, 3-CQA was degraded to undetectable level in all sweet potato samples. Also, 3,4-diCQA was degraded only in RGSP sample. The increasing rate of gallic acid was 9 times, 32%, 196%, and 152% in boiled RGSP, HSP, JeSP and JaSP samples, respectively. The 5-CQA and 4-CQA increased 84% and 38 times in RGSP sample, 108% and 16 times in JeSP sample and 154% and 41 times in JaSP sample, respectively. In boiled RGSP sample, the levels of 4,5-diCQA and 3,5-diCQA increased 51 and 9 times than its raw sample, respectively. In addition, 3,5-diCQA increased 13 times in boiled JeSP sample. Compared with the raw sample, 5-CQA, 4-CQA, 4,5-diCQA, and 3,5-diCQA became to detectable level in HSP sample. Similarly, 4,5-diCQA and 3,5-

diCQA could be identified in JaSP sample while 4,5-diCQA was detected in JeSP sample after boiling (Table 2.5). It was reported that after microwaving, boiling, and baking, the phenolics in sweet potato flesh would increase due to heat can induce CQAs isomerized (Padda and Picha, 2008b; Takenaka et al., 2006). According to the research by Joong-Keun Jung et al. (2011), the concentration of phenolics in Borami sweet potato also degraded at temperature 80 °C. The degradation of phenolic acids after boiling was due to a combination of leaching into water, degradation that is affected by heat, oxidation by polyphenol oxidase, and isomerization. Figure 2.15 is a typical chromatogram of phenolic acids in the raw sweet potato samples.

Table 2.4 Concentration of new phenolic acids in sweet potato samples

Concentration ( $\mu\text{g/g}$ )		N1	N2	N3
Raw	RGSP	ND	ND	ND
	HSP	ND	ND	ND
	JeSP	ND	ND	ND
	JaSP	ND	ND	ND
Boiled	RGSP	ND	ND	494.0 $\pm$ 13.3 <sup>a</sup>
	HSP	15.3 $\pm$ 0.7 <sup>a</sup>	13.4 $\pm$ 1.3 <sup>a</sup>	57.8 $\pm$ 1.8 <sup>b</sup>
	JeSP	34.8 $\pm$ 0.5 <sup>b</sup>	21.1 $\pm$ 6.6 <sup>ab</sup>	261.4 $\pm$ 9.2 <sup>c</sup>
	JaSP	52.0 $\pm$ 6.6 <sup>c</sup>	29.0 $\pm$ 8.5 <sup>b</sup>	255.2 $\pm$ 9.3 <sup>c</sup>

N1, N2, N3: New compounds after boiling; ND: not detected. RGSP: red garnet sweet potato; HSP: hannah sweet potato; JeSP: jewel sweet potato; JaSP: Japanese sweet potato. Means with different letters are significantly different at  $P < 0.05$ .

Table 2.5 Concentration of phenolic acids in sweet potato samples

Concentration ( $\mu\text{g/g}$ )		Gallic Acid	P1	P2	P3	P4	P5	P6
Raw	RGSP	33.8 $\pm$ 2.0 <sup>a</sup>	45.3 $\pm$ 1.9 <sup>a</sup>	1141.7 $\pm$ 19.5 <sup>a</sup>	10.5 $\pm$ 2.5 <sup>a</sup>	7.8 $\pm$ 0.2 <sup>a</sup>	53.5 $\pm$ 5.3 <sup>a</sup>	7.2 $\pm$ 1.7 <sup>a</sup>
	HSP	54.6 $\pm$ 7.5 <sup>b</sup>	64.8 $\pm$ 9.5 <sup>b</sup>	ND	ND	ND	ND	ND
	JeSP	66.5 $\pm$ 4.7 <sup>bd</sup>	134.6 $\pm$ 9.5 <sup>c</sup>	322.7 $\pm$ 20.3 <sup>b</sup>	10.9 $\pm$ 0.6 <sup>a</sup>	ND	23.6 $\pm$ 2.5 <sup>b</sup>	ND
	JaSP	34.1 $\pm$ 8.1 <sup>a</sup>	14.1 $\pm$ 0.6 <sup>d</sup>	30.0 $\pm$ 13.9 <sup>c</sup>	5.7 $\pm$ 0.6 <sup>b</sup>	ND	ND	ND
Boiled	RGSP	336.0 $\pm$ 25.5 <sup>c</sup>	ND	2107.4 $\pm$ 74.0 <sup>d</sup>	198.7 $\pm$ 1.4 <sup>c</sup>	410.3 $\pm$ 11.3 <sup>b</sup>	519.9 $\pm$ 18.9 <sup>c</sup>	ND
	HSP	72.2 $\pm$ 5.6 <sup>d</sup>	ND	148.0 $\pm$ 5.5 <sup>e</sup>	17.4 $\pm$ 0.3 <sup>d</sup>	53.9 $\pm$ 1.5 <sup>c</sup>	55.98 $\pm$ 1.5 <sup>a</sup>	ND
	JeSP	196.8 $\pm$ 23.1 <sup>e</sup>	ND	670.6 $\pm$ 75.2 <sup>f</sup>	201.2 $\pm$ 22.2 <sup>e</sup>	191.6 $\pm$ 11.5 <sup>d</sup>	339.4 $\pm$ 3.9 <sup>d</sup>	ND
	JaSP	85.8 $\pm$ 14.1 <sup>d</sup>	ND	493.0 $\pm$ 75.0 <sup>g</sup>	90.3 $\pm$ 6.0 <sup>f</sup>	243.8 $\pm$ 17.4 <sup>e</sup>	246.0 $\pm$ 8.8 <sup>e</sup>	ND

P1: 3-CQA (neochlorogenic acid); P2: 5-CQA (chlorogenic acid); P3: 4-CQA (cryptochlorogenic acid); P4: 4,5-diCQA (isochlorogenic acid); P5: 3,5-diCQA (isochlorogenic acid); P6: 3,4-diCQA (isochlorogenic acid); ND: not detected. RGSP: red garnet sweet potato; HSP: hannah sweet potato; JeSP: jewel sweet potato; JaSP: Japanese sweet potato. Means with different letters are significantly different at  $P < 0.05$ .

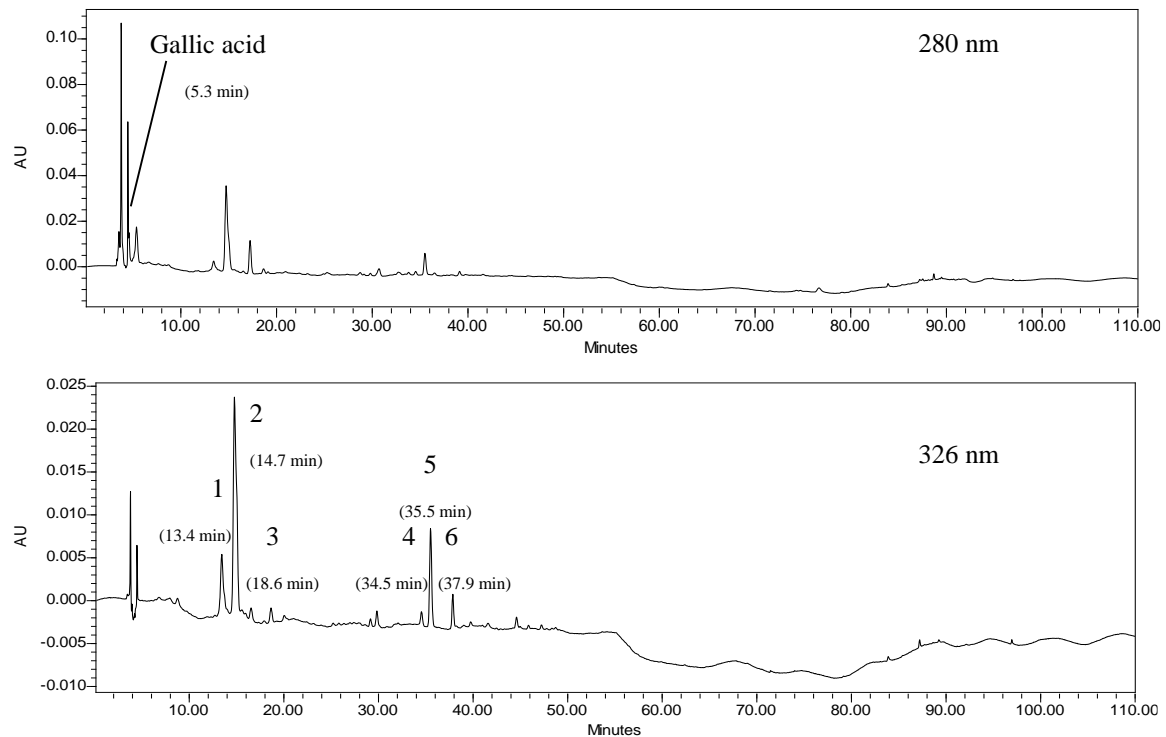


Figure 2.15 Typical chromatogram of phenolic acids in raw sweet potato extracts

- Peak1: 3-CQA (neochlorogenic acid);
- Peak2: 5-CQA (chlorogenic acid);
- Peak3: 4-CQA (cryptochlorogenic acid);
- Peak4: 4,5-diCQA (isochlorogenic acid);
- Peak5: 3,5-diCQA (isochlorogenic acid);
- Peak6: 3,4-diCQA (isochlorogenic acid).

A typical chromatogram of phenolic acids in the boiled sweet potato sample is showed in Figure 2.16. There were three types of new phenolic acids detected in the boiled sweet potatoes and expressed as N1, N2 and N3. The maximum absorbance wavelength of these new phenolic acids was at 328 nm. N1 and N2 could be found in the cooked potato samples and were caffeic acid derivatives. However, N3 was the unique phenolic acid which could only be detected in the boiled sweet potato sample. Boiled RGSP sample only had N3 while boiled HSP, JeSP and JaSP samples had all the three new compounds. Compared with N1 and N2, the concentration of N3 was the highest in boiled sample

among the sweet potato samples, especially in RGSP (Table 2.4). The reason for the differences of new produced compounds between the boiled potato and sweet potato samples may due to the varieties of phenolics and genotypes in their raw samples (Jung et al., 2011). Moreover, it is possible that some phenolic acids were changed into different isomers (Jung et al., 2011).

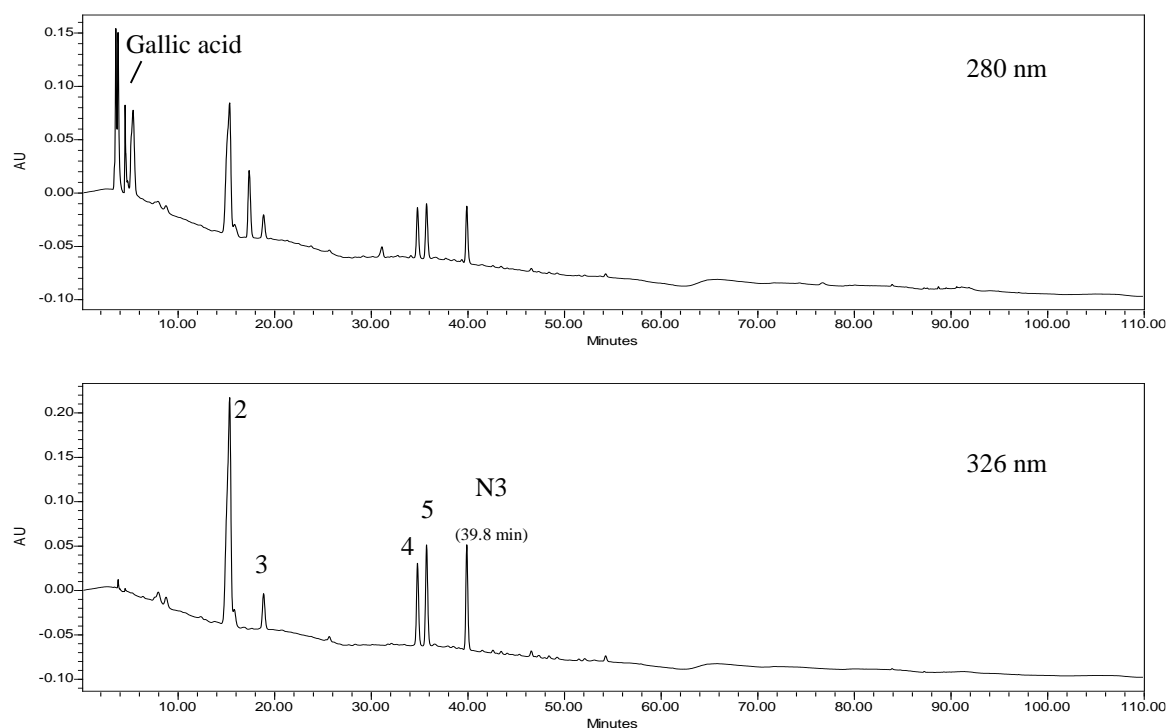


Figure 2.16 Typical chromatography of phenolic acids in the boiled sweet potato sample

Peak2: 5-CQA (chlorogenic acid);

Peak3: 4-CQA (cryptochlorogenic acid);

Peak4: 4,5-diCQA (isochlorogenic acid);

Peak5: 3,5-diCQA (isochlorogenic acid);

Peak6: 3,4-diCQA (isochlorogenic acid);

N3: new phenolic acid.



### 2.3.4. Comparison of Raw and Boiled Samples

The total concentration of phenolic acids increased significantly in each sample after boiling (Figure 2.17). Among these samples, the increasing rate of phenolic acids in RGSP sample was the highest. Phenolic acids are usually not stable after heat treatment. However, during heat treatment, bound phenolic acids could be released from the potato matrix more completely. Before heat treatment, only free phenolic acids were analyzed in samples. So the concentration of phenolic acids could increase after heating (Malmberg and Theander, 1984). Also, phenolic acids such as gallic acid and chlorogenic acids were not stable during thermal treatment (Friedman, 2000). Therefore, after boiling, gallic acids, 5-CQA, 4-CQA, 4,5-diCQA, and 3,5-diCQA increased while 3-CQA and 3,4-diCQA might reduce in the potato and sweet potato sample.

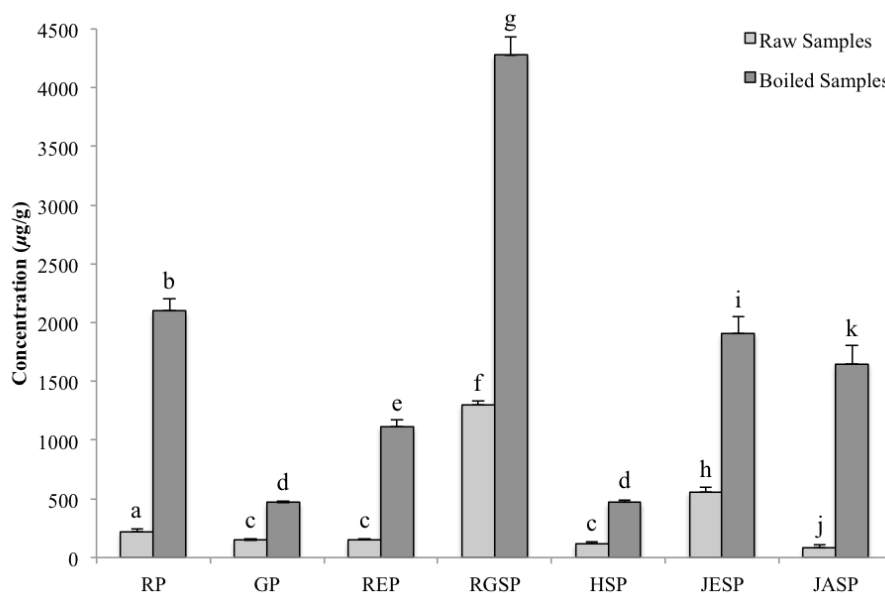


Figure 2.17 Total content of phenolic acids in the raw and boiled samples  
RP: loose russet potato; GP: loose gold potato; ReP: loose red potato; RGSP: red garnet sweet potato; HSP: hannah sweet potato; JeSP: jewel sweet potato; JaSP: Japanese sweet potato. Means with different letters are significantly different at  $P < 0.05$ .

## 2.4 Conclusion

In this study, seven individual phenolic acids, gallic acid, 3-CQA (neochlorogenic acid), 5-CQA (chlorogenic acid), 4-CQA (cryptochlorogenic acid), 4,5-diCQA (isochlorogenic acid), 3,5-diCQA (isochlorogenic acid) and 3,4-diCQA (isochlorogenic acid) were identified and quantified in the potato and sweet potato samples. Among the phenolic acids, 3,4-diCQA was only found in the sweet potato sample. Boiling treatment had significant effect on the level of each phenolic acid. After boiling, except for 3-CQA and 3,4-diCQA, the levels of all other phenolic acids increased. Moreover, the total concentration of phenolic acids in each sweet potato sample was much higher than that of each potato samples. Therefore, the sweet potatoes had higher level of antioxidants than the potatoes. The varieties of phenolic acids were wider in the sweet potatoes. Therefore, the sweet potatoes may have higher antioxidant activity than potatoes.

## CHAPTER 3. ANTIOXIDANT ACTIVITIES AND PHENOLIC ACIDS IN RED GARNET SWEET POTATO (RGSP) AT DIFFERENT BOILING TIME

### 3.1 Introduction

Sweet potato is a high-nutritious food containing carotenoids, phenolics and vitamins. The antioxidant activity of sweet potato was affected by the composition and concentration of phenolic acids (Javanmardi J. et al., 2003). The phenolic compounds in sweet potato are chlorogenic acid and other similar compounds (Agnes et al., 2007; Rudkin and Nelson, 1947). In general, phenolic metabolites are commonly found in fruits and vegetables for the protection from insect and animal attack (Asami et al., 2003). About 30% of dietary polyphenols are phenolic acids (Lall et al., 2015). As the major antioxidants in the sweet potato, phenolics are mainly influenced by the polyphenol oxidase which may cause degradation of the phenolics (Ahmed et al., 2011). It was reported that chlorogenic acids, 3,5-dicaffeoylquinic acid (3,5-diCQA), 3,4-dicaffeoylquinic acid (3,4-diCQA), and 4,5-dicaffeoylquinic acid (4,5-diCQA) extracted from steamed sweet potato can inhibit the production of melanogenesis in mice (Shimozono et al., 1996). Sweet potatoes have been reported to contain more isochlorogenic acid than other foods (Sondhermer, 1958).

As the seventh most important food crop in the world, sweet potato was commonly consumed after cooking (Li et al., 2012). The most common cooking method for sweet potato is boiling, however, this process may cause physical or chemical characters change in the sweet potato. Based on several previous studies, ascorbic acid and  $\beta$ -carotene

could be significantly reduced by different heat treatments, while the total phenolic contents increased (Dincer et al., 2011). The hydroxyl groups in phenolic compounds can donate hydrogen to lipid radicals to prevent lipid oxidation. Therefore, the phenolic acids in sweet potato have good antioxidant activity (Chaiyasit et al., 2007).

In this study, the changes of antioxidant activities and phenolics at different boiling times were measured. Since phenolic acids can affect not only the organoleptic properties (flavor, astringency, color), but also oxidative stability of the food, the results could be helpful in developing a cooking condition which can produce the cooked sweet potato with high antioxidant, flavor and color (Gruz et al., 2008). The objective of the research was to determine the antioxidant activity and concentration of phenolic acids in red garnet sweet potato at different boiling times and to obtain the best time for cooking sweet potatoes in order to yield the highest amounts of phenolic acids.

## **3.2 Material and Methods**

### **3.2.1 Sample Preparation for RGSP**

RGSP was purchased from a local “Whole Foods Market”, Baton Rouge. After peeled, the sweet potato were cut into small pieces and grounded by a kitchen blender with 400 ml distilled water until it became mash. It was poured into a beak and boiled. The beak was covered to avoid moisture loss. The mixture was collected at boiling time 0, 10, 20, and 30 min.

### 3.2.2 Moisture Analysis for Raw and Boiled Samples

The loss on drying (LOD) was used to determine the moisture content in each sample. Five gram of the sample was placed on a labeled foil dish. After dried at 80°C in an oven for six hours, the sample were reweighted and recorded to calculate the moisture content.

### 3.2.3 Extraction of Phenolics in RGSP

The collected samples were centrifuged to obtain the aqueous layer. The layer was transferred to a clean test tube for the DPPH, total phenolic content, and phenolic profile analysis (Figure 3.1).

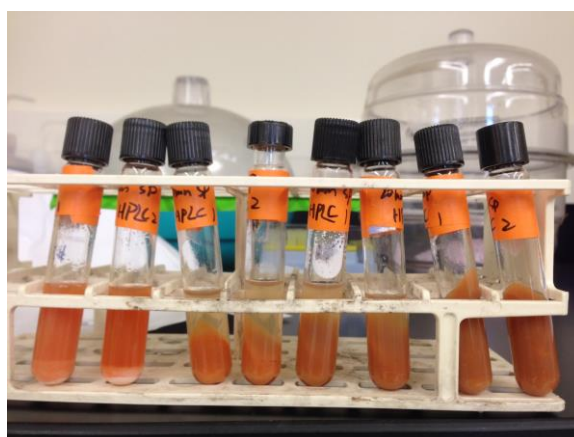


Figure 3.1 Boiled RGSP samples

### 3.2.4 DPPH Assay

The color change in DPPH assay was determined by the UV spectrophotometer shown in Figure 3.2. Five different concentrations of Trolox (0, 5, 10, 15, and 20  $\mu\text{g}/\text{mL}$ ) were used to prepare a standard curve. The test sample solution or Trolox (0.1 mL) was transferred into a cuvette and mixed with DPPH (0.1mM/L, 1.9 mL). The absorbance at wavelength

of 515 nm was recorded at 0 and 30 min, respectively. The free radical scavenging activity was expressed as  $\mu\text{g}$  Trolox equivalent /mL based on the standard curve.



Figure 3.2 UV Spectrophotometer

### 3.2.5 Analysis of Total Phenolics Content

The test sample solution (0.1 mL) was mixed with ten times diluted Folin–Ciocalteu reagent (0.75 mL) and incubated in dark for 5 min. Then, 0.75 mL of sodium bicarbonate solution (60 g/L) was added to the mixture and incubated for another 90 min. The absorbance of the mixture was measured at wavelength of 750 nm. A series concentration of catechin was used to set up a standard curve. The total phenolic content was expressed as  $\mu\text{g}$  catechin equivalent/mL based on the standard curve of catechin.

### 3.2.6 Phenolic Profile Analysis

An HPLC was equipped with a diode array detector (DAD) with wavelength scanning from 210 to 620 nm. The identification and quantification of phenolic acids was carried out by using a C<sub>18</sub> Column (250 × 4.6 mm). The wavelength was set at 280 nm for

quantifying gallic acid and other phenolic acids and 326 nm for chlorogenic acids and its derivatives. The extract solution (20  $\mu$ L) from each sample was injected into the HPLC system. The mobile phase consisted of 10% acetic acid (A) and acetonitrile (B). A gradient elution was employed with: 100% A, 0 min; 70% A, 50 min; 50% A, 70 min; 20% A, 80 min; 0% A, 85 min; 100% A, 90min and hold 20 min. The flow rate set at 0.8 mL/min.

### **3.3 Results and Discussion**

#### **3.3.1 Phenolic Acids in RGSP at Different Boiling Time**

Since the raw samples were mixed with distilled water during blending, the moisture contents were only used for adjusting the results obtained from each assay to be on dry basis. Gallic acid, five chlorogenic acids (3-CQA, 5-CQA, 4-CQA, 4,5-CQA and 3,5—CQA) and four new bioactive compounds (N1, N2, N3 and N4) were determined in the raw and cooked RGSP samples. The levels of all the original phenolics in the raw samples increased after boiling for 10 min and then decreased with the extended boiling time. The result is in accordance with antioxidant activity assays, in which, the highest antioxidant activity appeared at 10 min boiling time and then decreased.

In the raw sample, gallic acid, 3-CQA, 5-CQA, 4-CQA and three unknown phenolic acids (N1, N2 and N4) were determined. Gallic acid was determined at the wavelength of 280 nm and other phenolic acids were determined at 326 nm, except N4 which was at 360 nm.

The primary phenolic was 5-CQA was in all the samples followed by gallic acid and N2.

For samples boiled for 10 min and 20 min, gallic acids, other five phenolic acids (3-CQA, 5-CQA, 4-CQA, 4,5-CQA and 3,5-CQA) and four new phenolic acids (N1, N2, N3 and N4) were determined. However, 3-CQA decreased to not detectable level after boiled for 30 min. The samples boiled for 10 min maintained the most diversity profile and highest levels of phenolic acids (Figure 3.3). Chlorogenic acid isomers can be hydrolyzed to quinic acid and caffeic acid at boiling condition (Maldonado et al., 2014). However, it was reported that phenolic compounds can react with some minerals ( $\text{Cu}^{2+}$  and  $\text{Fe}^{3+}$ ) in the sweet potato to induce the prooxidant activity of phenolics (Figure 3.4) (Dai et al., 2010). The mechanism of the reactions between phenolic acids and minerals is demonstrated in Figure 3.4.

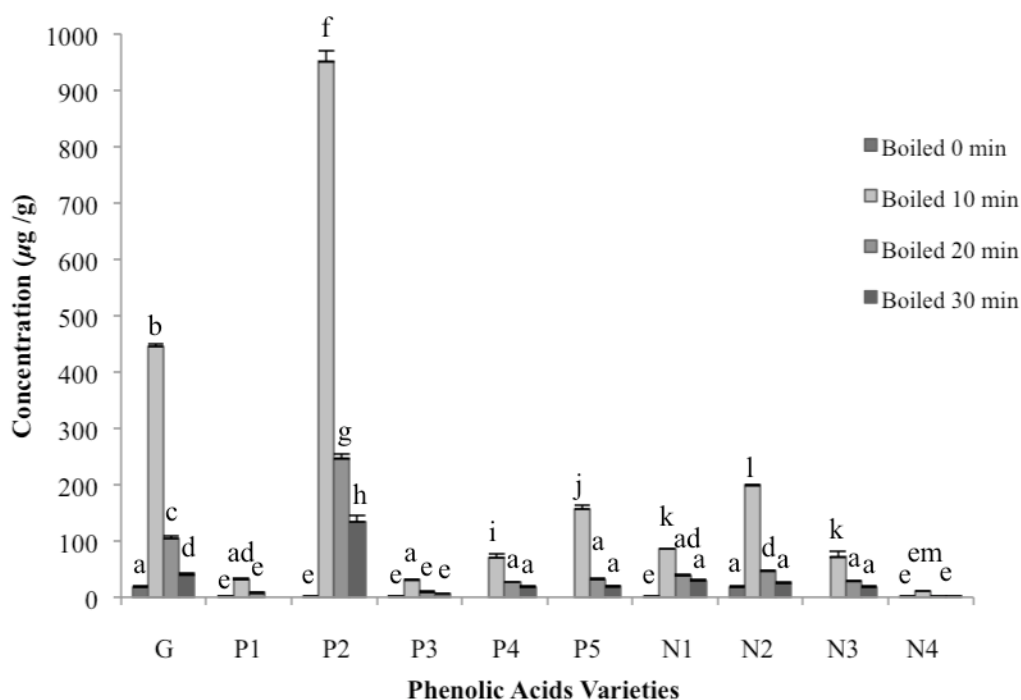


Figure 3.3 Concentrations of phenolic acids in RGSP at different boiling time  
 G: Gallic Acid; Peak1: 3-CQA (neochlorogenic acid); Peak2: 5-CQA (chlorogenic acid);  
 Peak3: 4-CQA (cryptochlorogenic acid); Peak4: 4,5-diCQA (isochlorogenic acid);  
 Peak5: 3,5-diCQA (isochlorogenic acid);  
 N1, N2, N3 and N4: New compounds after boiling.  
 Means with different letters are significantly different at  $P < 0.05$ .



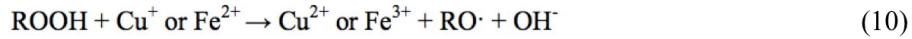
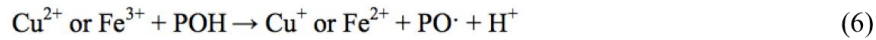
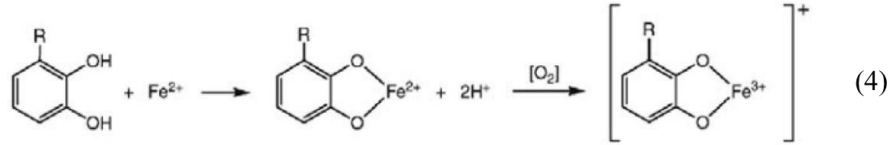
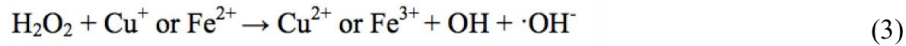


Figure 3.4 Reactions of phenolic acids with minerals (Jin Dai et al., 2010).

- (1) phenolic compounds acted with radicals;
- (2) free radicals forming;
- (3) metal ions interact with hydrogen peroxide to form hydroxyl radicals forming;
- (4) phenolic compounds with catechol and gallate groups react with metal ions;
- (5) forming of quinones;
- (6) prooxidant activity of phenolic antioxidants was induced by transition metal ions.

### 3.3.2 Antioxidant Activities in RGSP at Different Boiling Time

The free radical scavenging activities of all the samples were determined by using DPPH (1,1-diphenyl-2-picrylhydrazyl) assay. After 10 min of boiling, DPPH free radical scavenging activities in the boiled RGSP sample increased twice compared with the raw sample. However, the DPPH free radical scavenging activity decreased to the similar level as the raw sample after 30 min of boiling (Figure 3.5). Similar to the level of individual phenolic acid, the highest antioxidant activity occurred after 10 min of boiling. Since boiling treatment can release bound phenolic acids from the potato matrix, there were more free phenolic acids in the sample (Sosulski et al., 1982). The concentration of

phenolic acids can affect the antioxidant activity in the sample. The sample with higher concentration of phenolic acids would have higher antioxidant activity. After boiling, the hydrocarbon chains may break, making the phenolics more effective in inhibiting lipid oxidation and higher antioxidant activity (Sasaki et al., 2011). Moreover, the structure of phenolic acids can affect the antioxidant activity. For example, the 4,5-diCQA in sweet potato showed higher antioxidant activity than caffeoylquinic acid did. Therefore, the higher contents of 4,5-diCQA, the higher antioxidant activity would exhibit (Dini et al., 2006).

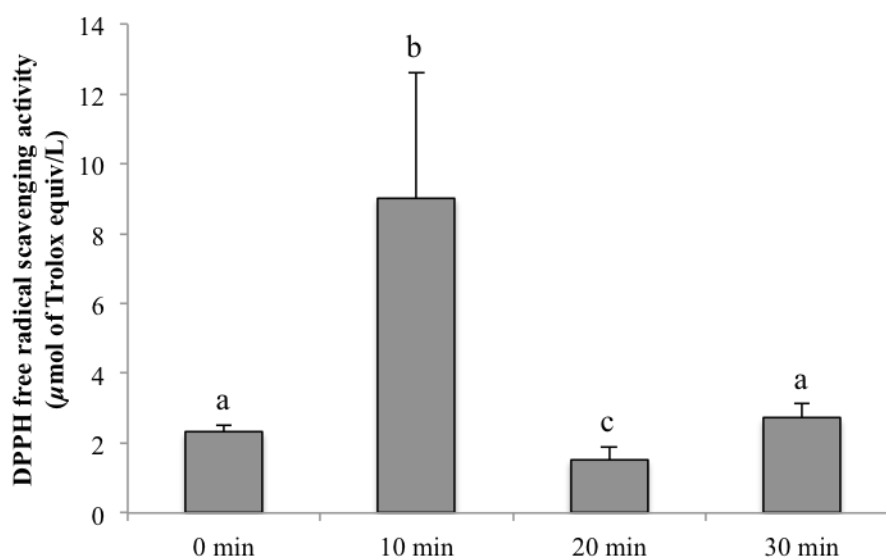


Figure 3.5 DPPH free radical scavenging activities of RGSP at different boiling time Means with different letters are significantly different at  $P < 0.05$ .

### 3.3.3 Total Phenolic Contents in RGSP at Different Boiling Time

Compared with the raw sample, the total phenolic content in cooked RGSP sample increased 4 times after 10 min of boiling and decreased with the extended boiling time (Figure 3.6). The increasing of total phenolic content was mainly due to the fact that bound phenolics were released from the sample matrix (Jacob et al., 2010). Generally, the antioxidant activity of a phenolic is dependent upon the number and position of hydroxyl groups. Since gallic acid has an extra hydroxyphenol group, the sample with more abundant gallic acid would have higher antioxidant activity (Elhamirad et al., 2012). Similar to the level of individual phenolic acid and DPPH free radical scavenging activities, the total phenolic content reached to the highest level after 10 min of boiling.

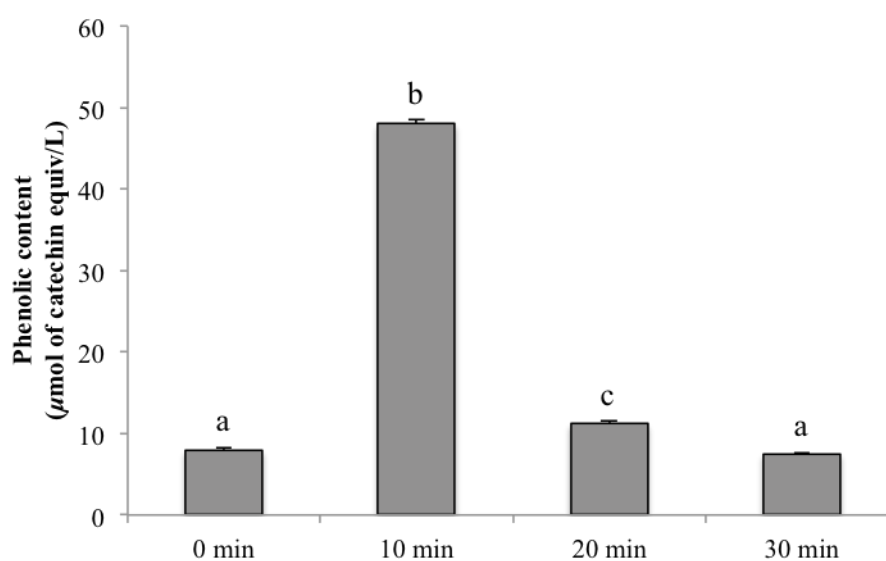


Figure 3.6 Phenolic contents of RGSP at different boiling time  
Means with different letters are significantly different at  $P < 0.05$ .

### 3.4 Conclusion

In this study, gallic acid and five varieties of chlorogenic acids: 3-CQA (neochlorogenic acid), 5-CQA (chlorogenic acid), 4-CQA (cryptochlorogenic acid), 4,5-diCQA (isochlorogenic acid) and 3,5-diCQA (isochlorogenic acid) were identified and quantified in cooked red garnet sweet potato. Each phenolic acid and antioxidant activity reached to the highest level in the cooked sweet sample after 10 min of boiling and decreased with continuous boiling. Furthermore, the DPPH free radical scavenging activities and total phenolic content of the cooked sweet potato were at the highest level after 10 min of boiling as well. However, the antioxidant activity decreased to the level similar to the raw sample after further boiling. Therefore, boiling for 10 min is the best time for the boiled sweet potato to have the highest health promoting activity compared.

## CHAPTER 4. SUMMARY

Phenolic compounds are important antioxidants in potatoes and sweet potatoes. The objectives of this study were to determine the differences of phenolic acids profiles between raw and boiled potatoes and sweet potatoes, as well as the effect of boiling time on the individual phenolic acid of sweet potato. In addition, the changes of antioxidant activity in the sweet potato during boiling were evaluated by the DPPH method. It was found that the level and diversity of phenolic acids varied by the varieties of potato and sweet potato samples. Moreover, it was found that 10 min of boiling was the best time for boiling blended sweet potato since its phenolic acid, DPPH free radical scavenging activity and total phenolic content reached the highest level. However, those levels decreased after the sweet potato sample was boiled more than 10 min. In addition, the phenolic acid content was in a positive correlation with the DPPH free radical scavenging activity. In general, gallic acid and 3,5-O-dicaffeoylquinic acid were the predominant phenolic acid in the raw potatoes and sweet potatoes, while 5-O-caffeoylquinic acid was the main phenolic acid in the boiled potatoes and sweet potatoes. Several new products generated during cooking could be the isomers of chlorogenic acid such as 3,4,5-O-tricaffeoylquinic acid which need to be further studied. The result of this research is useful for food industry in making sweet potato soup, sweet potato congee, mashed sweet potato, and sweet potato salad with high level of health promoting compounds.

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## VITA

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