

8-2010

# Effects of Common Culinary Cooking Techniques on the Antioxidants in Collard Greens

Alexander Clifford

Clemson University, [typetoalex@gmail.com](mailto:typetoalex@gmail.com)

Follow this and additional works at: [https://tigerprints.clemson.edu/all\\_theses](https://tigerprints.clemson.edu/all_theses)

 Part of the [Food Science Commons](#)

---

## Recommended Citation

Clifford, Alexander, "Effects of Common Culinary Cooking Techniques on the Antioxidants in Collard Greens" (2010). *All Theses*. 946.

[https://tigerprints.clemson.edu/all\\_theses/946](https://tigerprints.clemson.edu/all_theses/946)

This Thesis is brought to you for free and open access by the Theses at TigerPrints. It has been accepted for inclusion in All Theses by an authorized administrator of TigerPrints. For more information, please contact [kokeefe@clemson.edu](mailto:kokeefe@clemson.edu).

EFFECTS OF COMMON CULINARY TECHNIQUES ON THE ANTIOXIDANTS  
IN COLLARD GREENS

---

A Thesis  
Presented to  
the Graduate School of  
Clemson University

---

In Partial Fulfillment  
of the Requirements for the Degree  
Master of Science  
Food, Nutrition and Culinary Science

---

by  
Alexander Taylor Clifford  
August 2010

---

Accepted by:  
Dr. Paul Dawson, Committee Chair  
Dr. Anthony Pometto III  
Dr. Julia Sharp

## ABSTRACT

This thesis explores the effects of different cooking techniques on the antioxidant activity in collard greens. Chapter 1 is a literature review covering the topics of antioxidants, nutrition, cooking techniques and the effect of thermal treatment on antioxidants. Chapter 2 is a research manuscript evaluating collard greens from a local South Carolina farm exposed to seven different thermal treatments along with an untreated raw group. The thermal treatments utilized were sauté, both long and short simmer, the sauté treatment applied to both a long and short simmer treated group and the reserved cooking water from both a long and short simmer treatment group. After treatment, the total phenolic content (TPC) expressed in gallic acid equivalents/sample concentration (GAE/conc.) , 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferrous ion chelating (FIC) antioxidant assays were performed on all treatment groups. The sauté treated group showed the highest TPC (8.2858 GAE/conc.) followed by the raw group (8.0361) and the short simmer + sauté group (7.6227). The raw group showed the highest DPPH activity (7.7952 % inhibition/conc.) followed by the sauté group (7.5877) and the short simmer + sauté group (7.4753). In both of these assays the addition of a sauté treatment to either short or long simmered treatment increased the antioxidant activity of samples compared to just the short or long simmer treatment alone. Additionally both TPC and DPPH assays showed greater antioxidant activity in the cooking water reserved from a long simmer treatment compared to the reserved cooking water of a short simmer

treatment suggesting significant ( $p \leq 0.05$ ) leeching of antioxidants from collard greens into the water related to the duration of aquathermal treatment. Similar trends were not found in the results of the FIC chelating assay where both long and short simmer treatment groups showed the highest chelating abilities and the reserved cooking water from both treatments showed the lowest chelating abilities. This suggests that chelators contained in collard greens are not water soluble and therefore not negatively affected by aquathermal treatments.

## **ACKNOWLEDGEMENTS**

I would like to thank all of my professors and fellow students who helped me along the way. Throughout my years of poor health at Clemson, my professors never gave up on me, even when I mistakenly tried to give up on them. The faculty of the Food Nutrition and Culinary Science department is top notch and I am severely grateful to the massive amount of knowledge they have bestowed upon me. My Committee members, Dr. Dawson, Dr. Pometto III and Dr. Sharp have all been dearly supportive of me during this journey of knowledge and because of them I leave Clemson a better person than when I first arrived.

## TABLE OF CONTENTS

	Page
TITLE PAGE .....	i
ABSTRACT .....	ii
ACKNOWLEDGEMENTS .....	iv
Chapter 1: REVIEW OF ANTIOXIDANTS AND COOKING .....	1
Introduction .....	1
Antioxidants in Food Production .....	1
Antioxidants and Health .....	3
Effects of Cooking on Antioxidants .....	4
Other Factors on Antioxidant Profiles .....	8
Proper Cooking Techniques .....	9
Antioxidant Analysis.....	10
Conclusions .....	13
References .....	14
Chapter 2: DIFFERENT CULINARY TECHNIQUES AFFECT ANTIOXIDANT ACTIVITY OF COLLARD GREENS.....	18
Introduction .....	18
Materials and Methods.....	19
Results.....	26
Discussion .....	30

Table of Contents (Continued)

	Page
Conclusions .....	34
References .....	37

## LIST OF TABLES

	Page
Figure 1. TPC assay results with standard error bars .....	27
Figure 2. DPPH assay results with standard error bars .....	28
Figure 3. FIC assay results with standard error bars .....	29
Table 1. Total phenolic content (TPC), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferrous ion chelating capacity (FIC) of collard greens prepared using different cooking treatments.....	30
Figure 4. Results for TPC and DPPH assays .....	33
Figure 5. Relation of relative ranks of assay results for all assays.....	34

## Chapter 1: REVIEW OF ANTIOXIDANTS AND COOKING

### **Introduction**

Collard greens are a popular vegetable in comfort food and regional south cuisines with many different recipes available for preparation. The green can be found readily in many supermarkets across the southern United States throughout the year and comes from the *Brassica* family of vegetables, a genus known for its high nutritional value. Of great interest in the scientific field is the nutritional and preventative effects of antioxidants commonly found in high levels in fruits and vegetables. While collard greens may potentially contain beneficial antioxidant compounds, they are seldom consumed in their raw form and are subjected to different types of thermal treatment which may affect their overall nutritional and antioxidant value. The effect of cooking on the antioxidant activity of prepared collard greens may be an important factor because of the different methods of preparation.

### **Antioxidants in Food Preservation**

Antioxidants are of growing interest in recent years. More and more research is focusing on natural food antioxidants as the public is becoming aware of the importance antioxidants play in a healthy diet. The United States Department of Agriculture defines food antioxidants as “substances used to preserve food by retarding deterioration, rancidity or discoloration due to

oxidation.” Antioxidants are present in many types of food because of their important role in preventing oxidative deterioration of lipids by reacting with free radicals that are part of the oxidation process, chelating metal ions that can initiate autoxidation or being oxygen scavengers (Shahidi et al., 1992). Phenolic antioxidants are responsible for the majority of the oxygen capacity in most plant-derived products (Singleton et al., 1999).

Phenols include a wide array of compounds including flavones, tocopherols, hydroxycinnamites as well as many synthetic food antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), all sharing the presence of at least one phenol group (Singleton et al., 1999). Phenolics are capable of acting not only as radical scavengers but also as metal chelators and oxygen scavengers (Shahidi et al., 1992). They are a potent source of antioxidant activity and have been shown to be more effective at preventing lipid peroxidation than many vitamins (Rice-Evans, Miller and Paganga, 1997). In most cases, polyphenols, phenols with multiple phenol groups, exhibit greater antioxidant activity than monophenols (Brand-williams, Cuvelier and Berset, 1995). Some powerfully antioxidant plant source polyphenols include theaflavin digallate, epicatechin gallate, quercetin and coumaric-acid (Rice-Evans, Miller and Paganga, 1997).

In food systems, antioxidants are added in order to prevent or lessen textural and sensory changes of food items. Even small amounts of oxygen can

cause lipid peroxidation in food systems (Shahidi, Janitha and Wanasundara, 1992). As lipids undergo oxidation they can be degraded into volatile compounds that negatively influence the odor and flavor of food. Oxidative damage can also cause structural changes in lipids through the formation of conjugated dienes that result in alterations of texture and thermal stability (Smith, King and Min, 2007).

### **Antioxidants and Health**

In addition to protecting against quality degradation in food systems, antioxidants can have positive effects on overall human health. The addition of antioxidants into foods can help boost overall nutritive values with often positive effects on sensory quality (Skribic and Filipcev, 2008). In epidemiological studies it has been shown that consumption levels of fruits and vegetables rich in antioxidants and polyphenols is inversely related to a persons risk of having a stroke, death from a stroke, incidence of cardiovascular disease, cardiovascular disease mortality and overall mortality (Bazzano et al., 2002). The health benefits of plant-based antioxidants extend even further. Plant ethanol extracts have been shown to cause a decrease in lipid peroxidation in rat livers (Ardestani and Yazdanparast, 2007). Black poplar mushroom extracts were found to inhibit proliferation of stomach, breast and central nervous system cancers by over 23% *in vitro* (Diyabalanage et al., 2008). Plant extracts high in phenols have been

found to reduce growth in human breast cancer cells as well as reduce the toxicity of lung cancer cells (Yu et al., 2008).

Varietals of the plant species *Brassica oleracea* in particular have been shown to have positive health benefits possibly extending beyond just their antioxidant activities. Consumption of cabbage caused a significant reduction in breast cancer tumor formation in rats. Indole-3-carbinol was found to inhibit tongue carcinogens. Brussel sprouts consumption induces production of glutathione-S-transferase, an enzyme used to clear toxins from the body. Even after thermal treatment, Brassica extracts still showed antimutagenic activity. Plants from the Brassica genus have been shown to have a positive influence on preventing initiation of cancer as well as significantly reducing cancer cell proliferation and inducing natural production of enzymes which help the body fight cancer (Beecher, 1994).

### **Effect of Cooking on Antioxidants**

Cooking food emerged possibly as early as the Middle Pleistocene era (400,000-125,000 years ago) when some archeologists believe evidence exists of charred bones in hearths (Jurmain et al., 1999). While the cooking of produce is most often done for alteration of flavor and texture, cooking can lead to beneficial changes in edibility with the removal or inactivation of harmful or anti-nutritive components (Shahidi et al., 1992). Antioxidant assays of unprocessed food items may not accurately represent the health impact of foods that were

cooked or thermally processed prior to consumption. Different cooking methods can affect various indicators of antioxidant activity in produce. Total reducing capacity of vegetables can be significantly reduced by processing and thermal treatment. For a large part, this reduction is due to an overall loss via leeching of hydrophilic antioxidants while there is a relative increase in the lipophilic reducing capacity via concentration of non-hydrophilic compounds as a result of almost all thermal treatments. This increase in lipophilic reducing capacity may also be due to an increase in the bioavailability of lipophilic antioxidants or the development of Maillard reaction products that can exhibit antioxidant properties themselves (Greco et al., 2007).

Further evidence of the effect on the antioxidant profile of produce has been reported in some vegetables, like peppers, where the DPPH radical scavenging activity was not altered by either microwave cooking or stir-fry while boiling caused a significant decrease. In boiled samples, as time increased the amount of antioxidants leached into the boiling water also increased while the radical scavenging activity of vegetable samples decreased (Chuah, et al., 2008). Similarly, total phenolic content decreased in boiled pepper samples as boiling time increased while total phenolic values of the boiling water increased. When the ascorbic acid content of boiled pepper tissue and the boiling water were combined, they equaled the ascorbic acid content of raw pepper samples in those cultivars with thickened cell walls, while those with thin cell walls had a significant decrease in total ascorbic acid content occurring from boiling (Chauh

et al., 2008). Ferrous ion chelating (FIC) power of broccoli can also be affected by different types of thermal treatment. Mild thermal treatments have a minimal effect on broccoli's FIC power, while a more severe thermal treatment can reduce broccoli's chelating power. While these differences were shown with FIC power, DPPH radical scavenging activity showed insignificant differences between cooked and raw samples and also in comparison to BHA. As for anti-peroxidative effects, boiled broccoli exhibited greater ability to prevent linoleic acid peroxidation than fresh and lightly cooked broccoli (Lin and Chang, 2005). Furthermore, broccoli texture was inversely related to anti-peroxidative values, which relates to the raw versus cooked effect (Lin and Chang, 2005). Lipophilic antioxidant activity was reduced in kale, spinach and swamp cabbage when subjected to 1 minute of boiling. The same thermal treatment also caused a loss of total phenolic content (measuring both lipophilic and hydrophilic compounds) in kale, spinach, swamp cabbage as well as shallots and cabbage samples (Ismail, Marjan and Foong, 2004). Various thermal treatments including blanching, steaming and boiling all caused increased total folate concentration in broccoli samples compared to raw samples. Of these, steamed broccoli expressed the highest concentration of total folates. The same trends were not found in other vegetables tested such as potatoes where only sous-vide methods increased folate concentrations and in green peas where all of the thermal treatments caused a decrease in total folates. In all of the treatments, holding

time reduced total folate concentration in samples compared to their original values (Stea et al., 2006).

Boiling appears to have the most marked negative impact on antioxidant levels. Total phenolic content values were found to increase in steamed cabbage samples but decreased in boiled samples (Volden et al., 2008). Both steaming and boiling in legumes reduced total phenolic content and DPPH scavenging activity with the greater reduction in the boiled samples (Xu and Chang, 2008a). The temperature used in boiling also had an impact on antioxidant activity. The thermal effects on TPC of various Japanese vegetables were observed at various temperatures. While aquathermal treatment at 100 and 75°C caused a significant decrease in TPC of all samples, a 50°C treatment did not cause a reduction in vegetable TPC. The same trends were observed for DPPH radical scavenging activity. In some samples, a thermal treatment at 50°C for 10 minutes increased inhibition of hydroxyl radical activity compared to inhibition from raw controls (Roy et al., 2007).

The negative effect of aquathermal treatment on antioxidant profiles of produce may be partially due to leaching of antioxidants into the water. In thermally treated black beans, between 75 and 79% of phenolics were leached into the soaking and cooking waters (Xu and Chang, 2008b). The amount of antioxidants leached into cooking water increased as thermal treatment times increased (Wachtel-Galor, Wong and Benzie 2008). Because of this leaching

some studies recommend finding ways to minimize the amount of water used and reintroduce the cooking water into the final dish (Wachtel-Galorr, Wong and Benzie, 2008; Chuah et al., 2008).

Cooking foods can increase the extractability of various enzymes and polysaccharides that can both positive and negative effects on the nutritional content and antioxidant activity. Not only can this increase in enzyme availability cause an increase in activity of some beneficial compounds but it can also bind to beneficial molecules reducing their bioavailability (Sun-Waterhouse et al., 2008).

### **Other Factors on Antioxidant Profiles**

Antioxidant profiles of produce can vary due to many factors involved in the quality and condition of produce. Radical scavenging, metal chelating and total phenolics levels of kale, spinach, broccoli, carrots, tomatoes and potatoes differed from samples of the same vegetable grown on different farms or in different states (Zhou and Yu, 2006). Antioxidants in produce can vary depending on factors such as cultivar, genetics, maturity, growing conditions and environment. Elevated glucose levels associated with maturation could also affect a vegetable's antioxidant capacity (Chuan et al., 2008). For example total phenolics and ascorbic acid levels of guava fruit decreased with ripening as the tissues softened (Bashir and Abu-Goukh, 2003). The antioxidant profile of the

soil in which a plant is grown can also influence the plants antioxidant profile (Romani et al., 2003).

### **Proper Cooking Techniques**

Studies examining the cooking effects on antioxidant content in food often use boiling as the means of thermal treatment (Gayathri et al., 2004; Sikora et al., 2008; Ewald et al., 1999; Ferracane et al., 2008; Chu, Chang and Hsu, 2000). McGee's "*On Food and Cooking*" (1984) supports the use of boiling with vegetables, especially green vegetables that contain high levels of chlorophyllase, an enzyme active between 150°F-170°F that is responsible for altering chlorophyll making the molecule more prone to alteration. The high temperature of the boiling water is used to inactivate the enzyme in order to retain the bright green color of the vegetables while the water is useful for diluting plant acids that would also affect chlorophyll stability by a replacement of the magnesium ion with hydrogen atoms. In the official cookbook of The Culinary Institute of America "*The Professional Chef*" (2002), vegetables such as broccoli, spinach and collard greens are prepared by boiling, defined here as submersion in rapidly boiling water for at least two to three minutes and then drained. "*The Professional Chef*" also mentions that similar to boiling, parboiling is used for vegetables where they are prepared by liquid thermal treatment as per boiling yet are only cooked to partial doneness and then "finished by grilling, sautéing, or stewing". Alternatively, in an additional cookbook focusing on nutrition, "*The*

*Professional Chef's Techniques of Healthy Cooking*' states that boiling is not a recommended cooking method and that many times the term boiling, where the cooking liquid is around 212°F, is used when in reality the item is actually simmering, where cooking liquid is kept between 185 and 200°F. "*Techniques of Healthy Cooking*" also recommends dry heat cooking methods such as sautéing, grilling and baking or methods relying on steam over simmering or boiling as they "retain more water-soluble nutrients" (The Culinary Institute of America, 2000).

### **Antioxidant Analysis**

#### Total Phenolic Content

Phenols encompass the majority of plant-derived products antioxidant capacity. Both polyphenols and monophenols work as antioxidants in food systems, with polyphenols more readily oxidized than monophenols. Because both monophenols and polyphenols are important antioxidant systems, quantitative assays of phenols must account for both types (Singleton et al., 1999). Folin and Dennis (1912) along with Folin and Ciocalteu (1927) recommended reagents to determine through colorimetric means the total amount of phenols in aqueous solutions. Other competing phenolic assays are permanganate titration, ultraviolet absorbance and ferrous ion chelating however these are not recommended as they are either subject to interferences from sugars, not easily conducted using standard lab equipment or do not react with

monophenols. Comparison of the Folin-Dennis and Folin-Ciocalteu reagents (FDR, FCR) found the FCR to be a superior assay for total phenolic content as it does not suffer from formation of white precipitates, along with it being more sensitive of an analysis (Singleton et al., 1999). The reagent proposed by Folin and Ciocalteu is an intense yellow acidic solution of polyphosphotungstate-molybdate that is not stable under alkali conditions and fades rapidly to clear under high pH and high temperature (Folin and Ciocalteu, 1927). Phenols react more rapidly under alkaline conditions, so once reagent is thoroughly mixed with the test solution it is adjusted to a pH of around 10 and then observed after 2 hours at room temperature. Because of the differences in antioxidant abilities of different phenols standard solution comparisons such as gallic acid, tannic acid, catechin and tyrosine are often used. Gallic acid is the recommended standard solution because of low range of color yield per unit weight (Singleton et al., 1999).

#### DPPH

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) is a stable free radical used commonly in antioxidant capability analysis for its observed ability to be a hydrogen acceptor for antioxidants (Kurechi, Kikugawa and Kato, 1980). It absorbs strongly as a purple color at 517nm that fades to yellow as it interacts with hydrogen donating antioxidants (American Oil Chemists' Society, 1993). As

such, DPPH is used as a simple colorimetric assay of antioxidant capability. Reaction rates to DPPH have been shown to differ greatly over different types of antioxidants. Kurechi, et al. (1980) found tocopherol to reach a steady state in 5 minutes while they found BHT to require over 2 hours to reach steady state. Brand-Williams, et al. (1995) meanwhile found antioxidants such as ascorbic acid, isoascorbic acid and isoeugenol to react quite rapidly with DPPH, reaching steady state in their reactions in under 1 minute. Brand-Williams et al. (1995) also found rosmarinic acid to react in a similar time frame as tocopherol and found many antioxidants taking between 1 hour and 6 hours to reach a steady state in their reactions with DPPH. Even under optimal conditions not all antioxidants will react to diminish initial DPPH concentration by 100% as vanillin and coumaric acid never reached over 75% interaction.

### Ferrous Ion Chelating

Ferrozine is a reagent commonly used for the detection of ferrous ions in a solution. The reagent forms a stable complex with the ferrous ion that has a deep purple color absorbance peak at 562nm (Decker and Welch, 1990). This reagent can be used to test the chelating ability of a solution to bind to the reactive ferrous ion (Singh and Rajini, 2004). As a substance chelates iron from the ferrozine complex, absorbance at 562nm decreases. A reason for measuring the ferrous ion chelating ability of a solution is because of the ability of metal ions to

strongly produce singlet oxygen which can lead to increased propagation in lipid oxidation (Shahidi et al., 1992). Therefore a solution that showed a high chelating ability to ferrous ion will quench this oxidative process by preventing or reducing the formation of hydroxyl radicals (Chew et al., 2008). Ferrous salt colorimetry is useful in tests when potential interference from dextrin's, melanoidins and proteins is a concern (Singleton et al., 1999).

### **Conclusions**

Antioxidants such as those found in *Brassica* vegetables not only have useful applications to food processing but also are a beneficial part of the human diet. They provide molecular protection against oxidation as well as helping to prevent or battle various forms of cancer. Consumption of these vegetables however often times is precluded with the vegetables undergoing some form of thermal treatment. Previous research has shown that such thermal treatments can have differing effects on overall antioxidant values of the resulting vegetable dish. Previous research on thermal treatment of vegetables may not always look at the optimal form of cooking for vegetables and as such may provide results that are not entirely applicable to the common consumer. Observation on the effects of nutritionally sound cooking techniques on the antioxidant values of collard greens would further food science knowledge and provide important information for the proper preparation of this vegetable for the consumer.

## References

- American Oil Chemists' Society. 1993. Official and Tentative Methods of the American Oil Chemists' Society. Chicago, IL: American Oil Chemists' Society.
- Ardestani, A, Yazdanparast, R. 2007. Antioxidant and Free Radical Scavenging Potential of *Achillea santolina* Extracts. *Food Chemistry*. 104: 21-50.
- Diyabalantage, T, Mulabagal, V, Mills, G, DeWitt, DL, Nair, MG. 2008. Health-Beneficial Qualities of the Edible Mushroom, *Agrocybe aegerita*. *Food Chemistry*. 108: 97-102.
- Bashir, HA, Abu-Goukh, ABA. 2003. Compositional Changes During Guava Fruit Ripening. *Food Chemistry*. 80: 557-563.
- Bazzano, LA, He, J, Ogden, LG, Loria, CM, Vupputuri, S, Myers, L, Whelton, PK. 2002. Fruit and Vegetable Intake and Risk of Cardiovascular Disease in US Adults: the First National Health and Nutrition Examination Survey Epidemiologic Follow-Up Study. 76(1): 93-99.
- Beecher, CWW. 1994. Cancer preventive properties of varieties of *Brassica oleracea*: a review. *American Journal of Clinical Nutrition*. 59: 1166S-1170S.
- Brand-Williams, W, Cuvelier, ME, Berset, C. 1995. Use of a Free Radical Method to Evaluate Antioxidant Activity. *Food Science and Technology*. 28 (1): 25-30.
- Chew, YL, Lim, YY, Omar, M, Khoo, KS. 2008. Antioxidant Activity of Three Edible Seaweeds from Two Areas in South East Asia. *Food Science and Technology*. 41: 1067-1072.
- Chu, YH, Chang, CL, Hsu, HF. 2000. Flavonoid Content of Several Vegetables and their Antioxidant Activity. *Journal of the Science of Food and Agriculture*. 80: 561-566.
- Chuah, AM, Lee, YC, Yamaguchi, T, Takamura, H, Yin, LJ, Matoba, T. 2008. Effect of Cooking on the Antioxidant Properties of Coloured Peppers. *Food Chemistry*. 111: 20-28.
- The Culinary Institute of America. 2000. *The Professional Chef's Techniques of Healthy Cooking*. New York, NY: John Wiley & Sons.
- The Culinary Institute of America. 2002. *The Professional Chef 7<sup>th</sup> Edition*. New York, NY: John Wiley & Sons.
- Decker, EA, Welch, B. 1990. Role of Ferritin as a Lipid Oxidation Catalyst in Muscle Food. *Journal of Agricultural and Food Chemistry*. 38 (3): 674-677.

Diyabalanage, T, Mulabagal, V, Mills, G, DeWitt, DL, Nair, MG. 2008. Health-Beneficial Qualities of the Edible Mushroom, *Agrocybe aegerita*. *Food Chemistry*. 108: 97-102.

Ewald, C, Fjelkner-Modig, S, Johansson, K, Sjöholm, I, Akesson, B. 1999. Effect of Processing on Major Flavonoids in Processed Onions, Green Beans, and Peas. *Food Chemistry*. 64: 231-235.

Ferracane, R, Pellegrini, N, Visconti, A, Graziani, G, Chiavaro, E, Miglio, C, Fogliano, V. 2008. Effects of Different Cooking Methods on Antioxidant Profile, Antioxidant Capacity, and Physical Characteristics of Artichoke. *Journal of Agricultural and Food Chemistry*. 56: 8601-8608.

Folin, O, Ciocalteu, V. 1927. On Tyrosine and Tryptophane Determinations in Proteins. *Journal of Biological Chemistry*. 73: 627-650.

Folin, O, Denis, W. 1912. On Phosphotungstic-Phosphomolybdic Compounds as Color Reagents. *Journal of Biological Chemistry*. 12: 239-243.

Gayathri, GN, Platel, K, Prakash, J, Srinivasan, K. 2004. Influence of Antioxidant Spices on the Retention of B-carotene in Vegetables During Domestic Cooking Processes. *Food Chemistry*. 84: 35-43.

Greco, L, Riccio, R, Bergero, S, Del Re, AAM, Trevisan, M. 2007. Total Reducing Capacity of Fresh Sweet Peppers and Five Different Italian Pepper Recipes. *Food Chemistry*. 103: 1127-1133.

Ismail, A, Marjan, ZM, Foong, CW. 2004. Total Antioxidant Activity and Phenolic Content in Selected Vegetables. *Food Chemistry* 87: 581-586.

Jurmain, R, Nelson, H, Kilgore, L, Trevathan, W. *Introduction to Physical Anthropology* 8<sup>th</sup> Edition. Belmont, CA: Wadsworth.

Kurechi, T, Kikugawa, K, Kato, T. 1980. Studies on the Antioxidants XIII Hydrogen Donating Capability of Antioxidants to 2,2-Diphenyl-1-picrylhydrazyl. *Chemical Pharmaceutical Bulletin*. 28 (7): 2089-2093.

Lin, CH, Chang, CY. 2005. Textural Change and Antioxidant Properties of Broccoli Under Different Cooking Treatments. *Food Chemistry*. 90: 9-15.

McGee, H. 1984. *On Food and Cooking*. New York, NY: Scribner.

Rice- Evans, CA, Miller, NJ, Paganga, G. 1997. Antioxidant Properties of Phenolic Compounds. *Trends in Plant Science*. 2 (4): 152-159.

Romani, A, Pinelli, P, Galardi, C, Corti, G, Agnelli, A, Vincieri, FF, Heimler, D. 2003. Flavonoids in Leaves of Black, Cabbage (*Brassica oleracea* var. *acephala*)

- dc. subvar. *Viridis* cv. *Serotina*). Grown on Different Soils and at Different Elevations. *Italian Journal of Food Science*. 2 (15): 197-205.
- Roy, MK, Takenaka, M, Isobe, S, Tsushida, T. 2007. Antioxidant Potential, Anti-Proliferative Activities, and Phenolic Content in Water-Soluble Fractions of Some Commonly Consumed Vegetables: Effects of Thermal Treatment. *Food Chemistry*. 103: 106-114.
- Shahidi, F, Janitha, PK, Wanasundara, PD. 1992. Phenolic Antioxidants. *Critical Reviews in Food Science and Nutrition*. 32(1): 67-103.
- Sikora, E, Cieslik, E, Leszczynska, T, Filipiak-Florkiewicz, A, Pisulewski, PM. 2008. The Antioxidant Activity of Selected Cruciferous Vegetables Subjected to Aquathermal Processing. *Food Chemistry*. 107: 55-59.
- Singh, N, Rajini, PS. 2004. Free Radical Scavenging Activity of an Aqueous Extract of Potato Peel. *Food Chemistry*. 85: 611-616.
- Singleton, VL, Orthofer, R, Lamuela-Raventos, RM. 1999. Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. *Methods in Enzymology*. 299: 152-178.
- Skrbic, B, Filipcev, B. 2008. Nutritional and Sensory Evaluation of Wheat Breads Supplemented with Oleic-Rich Sunflower Seed. *Food Chemistry*. 108: 119-129.
- Smith, SA, King, RE, Min, DB. 2007. Oxidative and Thermal Stabilities of Genetically Modified High Oleic Sunflower Oil. *Food Chemistry*. 102: 1208-1213.
- Stea, TH, Johansson, M, Jagerstad, M, Frolich, W. 2006. Retention of Foliates in Cooked, Stored and Reheated Peas, Broccoli and Potatoes for Use in Modern Large-Scale Service Systems. *Food Chemistry* 101: 1095-1107.
- Sun-Waterhouse, D, Smith, BG, O'Connor, CJ, Melton, LD. 2008. Effect of Raw and Cooked Onion Dietary Fibre on the Antioxidant Activity of Ascorbic Acid and Quercetin. *Food Chemistry*. 111: 580-585.
- Volden, J, Borge, GIA, Bengtsson, GB, Hanse, M, Thygesen, IE, Wicklund, T. 2008. Effect of Thermal Treatment on Glucosinolates and Antioxidant-Related Parameters in Red Cabbage (*Brassica oleracea* L. ssp. *Capitata* f. *rubra*). *Food Chemistry*. 109: 595-605.
- Wachtel-Galor, S, Wong, KW, Benzie, IFF. 2008. The Effect of Cooking on Brassica Vegetables. *Food Chemistry*. 110: 706-710.
- Xu, BJ, Chang, SKC. 2008a. Effect of Soaking, Boiling, and Steaming on Total Phenolic Content and Antioxidant Activities of Cool Season Food Legumes. *Food Chemistry*. 110: 1-13.

Xu, BJ, Chang, SKC. 2008b. Total Phenolic Content and Antioxidant Properties of Eclipse Black Beans (*Phaseolus vulgaris* L.) as Affected by Processing Methods. *Journal of Food Science*. 73 (2): H19-H26.

Yu, L, Zhao, M, Wang, JS, Cui, C, Yang, B, Jiang, Y, Zhao, Q. 2008. Antioxidant, Immunomodulatory and Anti-Breast Cancer Activities of Phenolic Extract from Pine (*Pinus massoniana* Lamb) Bark. *Innovative Food Science and Emerging Technologies*. 9: 122-128.

Zhou, K, Yu, L. 2006. Total Phenolic Contents and Antioxidant Properties of Commonly Consumed Vegetables Grown in Colorado. *Food Science and Technology*. 39: 1155-1162.

## Chapter 2: DIFFERENT CULINARY TECHNIQUES AFFECT ANTIOXIDANT ACTIVITY OF COLLARD GREENS

### **Introduction**

Antioxidants continue to be a focus in research, marketing and nutrition. While synthetic antioxidants are available to supplement processed foods in order to mediate the effects of lipid peroxidation, there are many natural sources available containing antioxidants (Moure et al., 2001). The benefits of antioxidants extend beyond just enhancing shelf life of products. Antioxidants are also highly prized by consumers for health benefits (Bazzano et al., 2002). From potentially reducing cancer risks, cardiovascular disease and Type II diabetes, antioxidants are drawing attention in food and diet.

There are different antioxidant assays available to determine the antioxidant content or power of foods. Tests can be direct in assessing a samples concentration of antioxidant compounds or alternatively can determine a samples ability to reduce a stable free radical or chelate a metal both of which can initiate or propagate the lipid peroxidation process (Prior, et al., 2005).

The antioxidant content or bioavailability of a product is not constant due to external factors. Thermal treatment is common in both processing and home preparation of foods. Studies have shown that thermal treatment can have an effect on the bioavailability and strength of a food product's antioxidants (Volden et al., 2009; Smith, et al., 2007; Singh and Rajini, 2004) and has been well

documented in different varieties of the *Brassica oleracea* species (Gazzani et al., 1998; Lisiewska et al., 2008; Volden et al., 2008; Roy et al., 2007; Wachtel-Galor, et al., 2008). Methods of thermal treatments in these studies are often-times simplified cooking applications that are seldom used by the home cook, making the results of these studies potentially not applicable to food service as well as the home setting. Temperatures obtained in home cooking are capable of altering enzymatic content of vegetables (McGee, 1984) and some methods such as simmering and boiling can diminish nutritional content (The Culinary Institute of America, 2000).

The objective of this study was to determine how commonly used cooking treatments can affect the antioxidant profile of a standard local crop (collard greens) from the well-studied *Brassica oleracea* species.

### **Materials and Methods**

Reagents used for the various tests were 3,4,5-trihydroxybenzoic acid (gallic acid (Sigma-Aldrich)), sodium bicarbonate (Sigma-Aldrich), 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma-Aldrich), 5,6-diphenyl-1,2,4,ferrozine (Sigma-Aldrich), FeCl<sub>2</sub> 98% (ferrous chloride (Sigma-Aldrich), Folin-Cocalteau Reagent (2N FCR (Sigma-Aldrich)) and Ethyl Alcohol 200 Proof (Deacon Laboratories Inc.) Distilled water was obtained from a Nanopure Infinity Ultrapure Water System unit (Barnstead).

All spectrophotometric analysis was performed on a Model 4001/4 Spectronic 20 Genesys (Spectronic Instruments). Precision mass measurements were recorded from a College B204-S model Monobloc analytical balance (Mettler Toledo). Precision volumetric measurements were done using either a 1-5ml Fisherbrand Finnpipette adjustable pipetter (Fisher Scientific) or a 1000 microliter reference adjustable pipetter (Eppendorf).

Fresh collard greens were purchased from a local supermarket. All collard greens were of the same shipment from a local supplier in South Carolina, Walter P Rawl and Sons, Inc and were grown in Gilbert, South Carolina.

### Sample Treatments

Collard greens were purchased while still under refrigeration, transported to the lab and wrapped in moist towels then stored at 3° C until ready for use and were used within one week of purchase. Experiment was conducted in a Randomized Complete Block Design. Each block consisted of one head of collard greens. To prepare each head of collard greens, the leafy portions were removed from the thick stalks and triple soaked in a vegetable preparation sink with cold water. Once collard greens were air dried in a large colander for 30 minutes, they were sliced into approximately one-eighth to one-quarter inch wide ribbons using the culinary chiffonade technique where the leaves are rolled crosswise and then sliced in the crosswise with an 8" Henkel Chef Knife. Twenty-

five gram samples of cut collard greens were chosen through simple random sampling from the sliced head of collard greens and assigned to one of eight treatments whose order had been randomly created for each different block; control, short simmer, short simmer reserved water, short simmer + sauté, sauté, long simmer, long simmer reserve water and long simmer + sauté. Water for simmering was prepared ahead of time in a large 4qt stock pot and kept at a steady 82°C.

If assigned to the control group, the sample was placed in a plastic bag and immediately placed under refrigeration at  $3^{\circ} \pm 2^{\circ}\text{C}$ . The remaining treatments were as follows: Short simmer; samples were added to 250ml of deionized water in a 1-qt sauce pan heated to 82°C and cooked for 5 minutes then drained in a colander for 10 minutes, stored in a plastic bag and immediately placed under refrigeration at  $3^{\circ} \pm 2^{\circ}\text{C}$ . Short simmer reserved water; collard greens assigned to this group were cooked identically to the short simmer method however the drained water was reserved and stored while the collard greens were then disposed and the water held for analysis at  $3^{\circ} \pm 2^{\circ}\text{C}$ . Short simmer + sauté; collard greens samples were cooked according to the short simmer method and after draining for 10 minutes in a colander, were further cooked in an 8" sauté pan with 50ml of 82°C deionized water over medium heat for 4 minutes before being bagged and stored identically to all solid treatment samples. Sauté; collard green samples were cooked in an 8" sauté pan over medium heat for 4 minutes with 50ml of 82°C deionized water before baggage and storage. Long simmer;

collard green samples were cooked in 250ml of 82°C deionized water for 20 minutes in a covered 1-qt sauce pan, then drained for 10 minutes in a colander, bagged and stored. Long simmer reserved water; samples assigned to this group were cooked identically to the long simmer treatment group and as with the short simmer reserved water group, the drained liquid was reserved for analysis, while the solids were then disposed. For the final treatment (long simmer + sauté), collard green samples were cooked according to the long simmer method before being drained in a colander for 10 minutes and then undergoing the sauté treatment method.

### Sample Extractions

Extraction procedures were based on Lim, et al (2007). For all but the reserved simmer water treatments, 6-10g of each sample were added to a blender with 100ml of 50% ethanol (v/v) and homogenized for 60 seconds. The homogenized mixture was further agitated for 10 minutes at 100 rpm with an Orbital Shaker model 3520 (Lab-Line/Thermo Fischer Scientific. Waltham, Massachusetts). Each mixture was then filtered through #40 filter paper (Whatman. Florham Park, New Jersey). Once yield was assessed volumetrically, 50ml of each sample was reserved and stored for no longer than one week at - 20° C.

## Solids Determination

The oven drying method was used to determine solids content. Approximately 2-3g of post-treatment collard greens were added to marked, dried and pre-weighed aluminum trays. Filled and weighed trays were placed into a model 130DM Thelco Laboratory Convection Oven (Precision Scientific/Thermo Fischer Scientific, Waltham, Massachusetts) and heated at 150°C. After 24 hours trays were equilibrated to room temperature in a desiccator, weighed again and sample moisture percentage was determined according to the following formula:

Solids (%)

$$= (T_{D1}-T_1)/(T_{F1}-T_1) \times 100\%$$

where  $T_{D1}$  is the mass of the oven dried tray with sample,  $T_1$  is the premeasured mass of the empty tray and  $T_{F1}$  is the mass of the filled pre-oven dried tray with sample. Solids percentage was then used to express each sample concentration in mg/ml format.

## DPPH Assay

DPPH assay procedures were based on (Ardestani and Yazdanparast, 2007). For each reading, 150  $\mu$ l of sample were added to 850  $\mu$ l of 50% (v/v) ethanol. To this 1 ml of 0.2mM DPPH in 50% (v/v) ethanol solution was added.

Mixtures were then vortexed and held for 30 minutes at room temperature after which, absorbance readings were recorded at 517nm. Absorbance readings were compared to a negative control of 50% (v/v) ethanol and expressed as a percentage change per sample concentration (mg/ml) according to the following formula:

Free radical scavenging activity (%/mg/ml)

$$= [(A_0 - A_1) / A_0] \times 100\% / \text{Conc}_1$$

where  $A_0$  is the absorbance of the control,  $A_1$  is the absorbance of sample and  $\text{Conc}_1$  is the mg/ml concentration of the sample.

#### Total Phenolics Assay

Total phenolic content was measured according to the Folin-Ciocalteu reaction (Singleton et al., 1999). Briefly, 200  $\mu\text{l}$  of sample were added to 1.00 ml of Folin-Ciocalteu reagent and resulting mixture was vortexed for five seconds then held for 1 minute. To this mixture, 800 microliters of 7.5%  $\text{NaHCO}_3$  was added then vortexed for 5 seconds and allowed to react for 15 minutes before absorbance was read at 765nm. A standard curve was obtained using a freshly made gallic acid solution with varying degrees of concentration ranging from 0-300 mg/ml in 50% EtOH and reacting in place of samples. Using the resulting standard curve ( $R^2=0.998$ ) with a slope of 0.006 and a y-intercept of 0.016

( $y=0.006x+ 0.016$ ), sample absorbance readings were converted into Gallic Acid Equivalents (GAE) and expressed in terms of GAE/mg/ml of sample according to the formula:

Gallic Acid Equivalents

$$=[(A_1-0.016)\times 0.006]/\text{Conc}_1$$

where  $A_1$  is the absorbance of the sample and  $\text{Conc}_1$  is the concentration of sample.

#### Ferrous Ion Chelating Assay

Chelating ability of the samples was determined by the ferrous ion chelating assay (Gulcin et al., 2008). From each of the sample extracts, 0.4ml was taken and mixed with 0.2ml of 2mM  $\text{FeCl}_2$ . To this 3ml of 50% ethanol was added and then reaction was initiated by addition of 0.4ml of 5mM ferrozine. The mixture was allowed to sit at room temperature ( $\sim 24^\circ \text{C}$ ) for 10 minutes before absorbance was measured at 562nm. A negative control of 50% (v/v) ethanol without sample was used for calculations. The samples ability to prevent formation of ferrozine- $\text{Fe}^{2+}$  complex was reported in relation to samples dry mass by using the formula:

Ferrous ion chelating activity (%/mg/ml)

$$=[(A_0-A_1)/A_0] \times 100\% / \text{Conc}_1$$

where  $A_0$  is the absorbance of the control,  $A_1$  is the absorbance of sample and  $\text{Conc}_1$  is the mg/ml concentration of the sample.

### Statistical Analysis

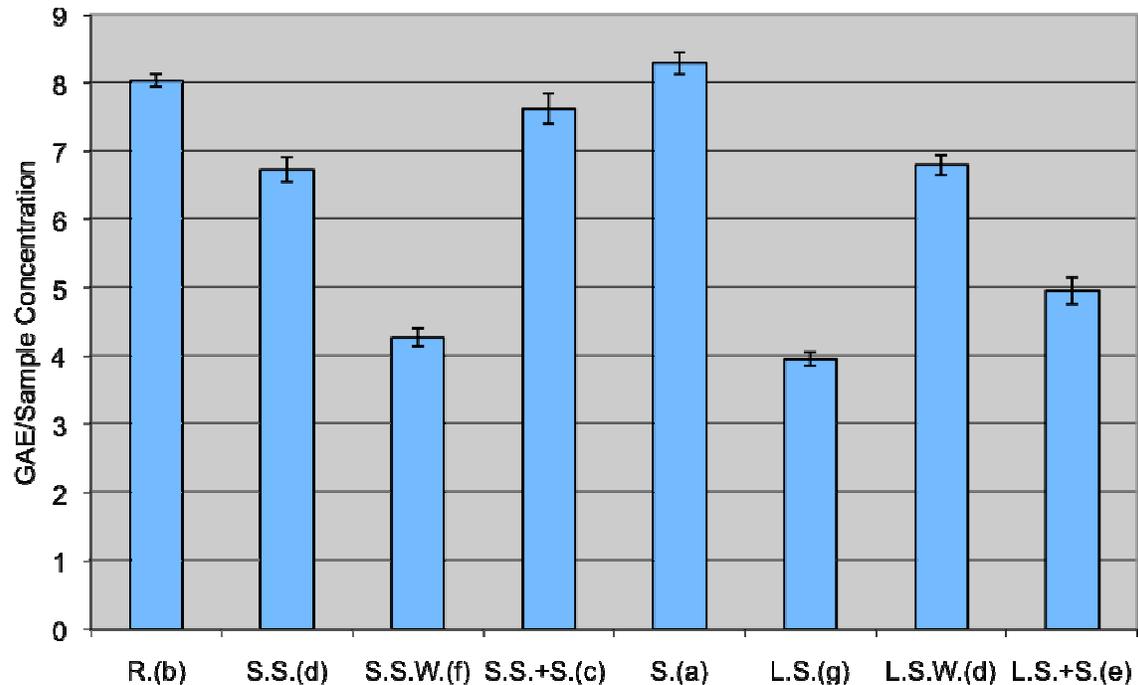
A Randomized Complete Block Design was used for assigning samples to treatment groups. Statistical analysis was carried out in SPSS release 15.0.0 using the General Linear Model univariate analysis with assigned blocks and treatments with a Fisher's Least Significant Difference post hoc test to determine significant differences ( $p \leq 0.05$ ) between treatments.

## **Results**

### Total Phenolics

Cooking method resulted in differences in TPC for collard greens with the sautéed collard greens containing the highest amount of gallic acid equivalents (Figure 1). The Raw (R.) followed by the Short simmer + sauté (S.S.+S.) treatments contained the next highest TPC. The Long simmer water (L.S.W.) was not different from the Short simmer group (S.S.) ( $p=0.246$ ). Gallic acid equivalents descended through the remaining treatments of Long simmer + sauté (L.S.+S.), Short simmer water (S.S.W.) and Long simmer (L.S.). All but the Long simmer water and Short simmered greens were found to be different ( $p \leq 0.05$ ) from other groups showing a total phenolics strength in the order listed above.

Figure 1. TPC assay results with standard error bars

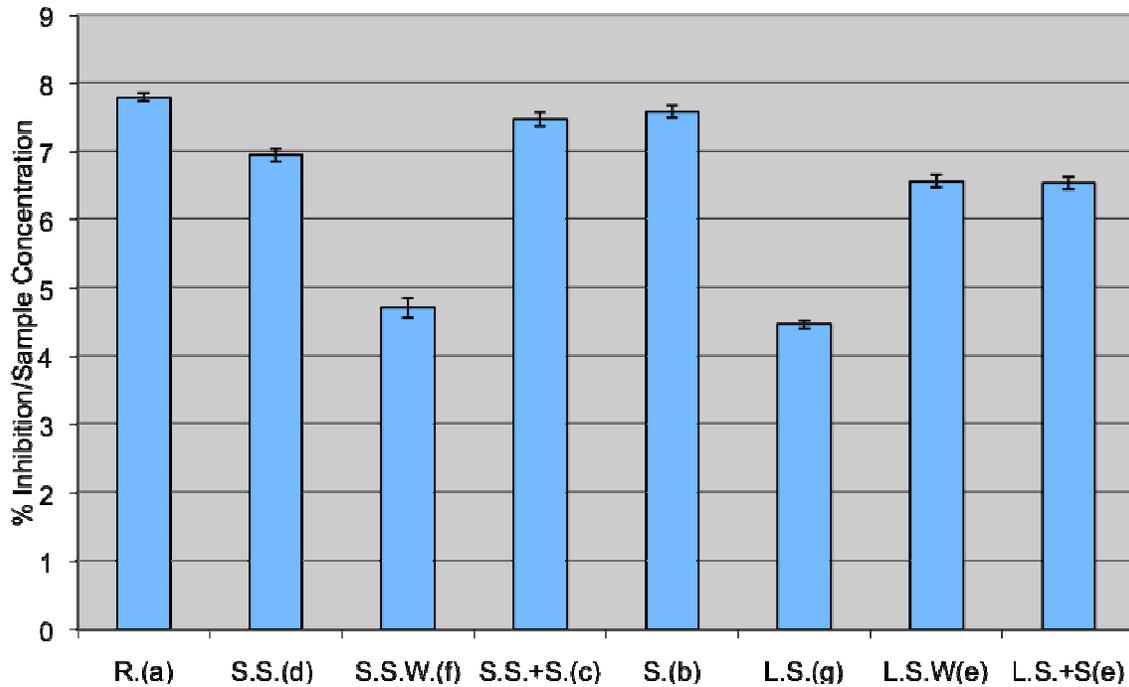


<sup>(a-g)</sup> means with different letters within columns are significantly different ( $p \leq 0.05$ ).  
 TPC = total phenolic content (Gallic acid equivalents/mg/ml)

#### DPPH

The DPPH assay showed similar results in ranking of treatments as the TPC assay. The order of the groups from strongest radical scavenging effect to lowest was Raw, Sautéed, Short simmer + sauté, Short simmer, Long simmer water and Long simmer + sauté, Short simmer water and lastly Long simmer (Figure 2). Treatments not differing in DPPH activity were the Long simmer water and Long simmer + sauté groups ( $p=0.590$ ). All other groups were significantly different at ( $p \leq 0.05$ ).

Figure 2. DPPH assay results with standard error bars

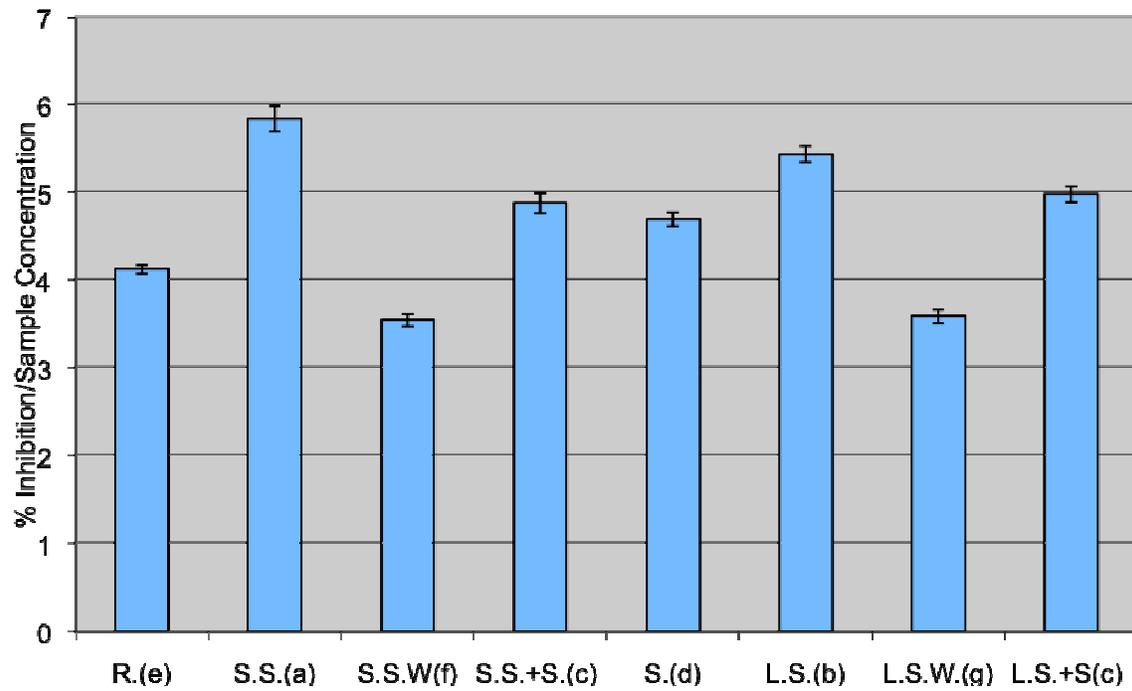


<sup>a-g</sup> means with different letters within columns are significantly different ( $p \leq 0.05$ ).  
 DPPH = 1,1-diphenyl-2-picrylhydrazyl (% change in absorbance/mg/ml)

### FIC

Results for the Ferrous Ion Chelating assay showed different trends compared to the other two assays. The treatment that was able to chelate the greatest amount of  $\text{Fe}^{2+}$  was the Short simmer (Figure 3). The next different treatment was the Long simmer. The Long simmer + sauté and the Short simmer + sauté did not differ ( $p=0.083$ ) in chelating capacity. The fourth and fifth best chelating treatments were the Sauté and Raw groups, respectively. The remaining two treatments of Long simmer water and Short simmer water had the lowest chelating capacity and did not significantly differ from each other ( $p=0.409$ ).

Figure 3. FIC assay results with standard error bars



<sup>(a-g)</sup> means with different letters within columns are significantly different ( $p \leq 0.05$ ).

FIC = ferrous ion chelating capacity (% change in absorbance/mg/ml)

Table 1. Total phenolic content, 1,1-Dipheynl-2-picrylhydrazyl (DPPH) and ferrous ion chelating capacity of collard greens prepared using different cooking treatments.

Treatment	TPC	DPPH	FIC
Raw	8.03 <sup>b</sup>	7.79 <sup>a</sup>	4.12 <sup>e</sup>
Short Simmer	6.73 <sup>d</sup>	6.94 <sup>d</sup>	5.84 <sup>a</sup>
Short Simmer Water	4.27 <sup>f</sup>	4.70 <sup>f</sup>	3.54 <sup>f</sup>
Short Simmer+Sauté	7.62 <sup>c</sup>	7.47 <sup>c</sup>	4.87 <sup>c</sup>
Sauté	8.28 <sup>a</sup>	7.58 <sup>b</sup>	4.69 <sup>d</sup>
Long Simmer	3.95 <sup>g</sup>	4.46 <sup>g</sup>	5.43 <sup>b</sup>
Long Simmer Water	6.79 <sup>d</sup>	6.56 <sup>e</sup>	3.58 <sup>f</sup>
Long Simmer+Sauté	4.95 <sup>e</sup>	6.53 <sup>e</sup>	4.97 <sup>c</sup>

<sup>a-g</sup> means with different letters within columns are significantly different ( $p \leq 0.05$ ).

TPC = total phenolic content (Gallic acid equivalents/mg/ml)

DPPH = 1,1-diphenyl-2-picrylhydrazyl (% change in absorbance/mg/ml)

FIC = ferrous ion chelating capacity (% change in absorbance/mg/ml)

## Discussion

The order of antioxidant strength of cooking treatments for both the total phenolics and DPPH assays followed a similar trend (Figure 4). In both cases the weakest group was Long simmer, followed by Short simmer water and then Long simmer + sauté. In both assays the two strongest groups were Sauté and Raw and the third strongest treatment in both assays was Short simmer + sauté.

Similar relationships between TPC and DPPH assays have been found by other researchers (Chew et al., 2008; Sikora et al., 2008; Xu, and Chang, 2008; Roy et al., 2007). Other positively correlated values between antioxidant tests have been shown in vegetables with TPC, Trolox Equivalent Antioxidant Capacity

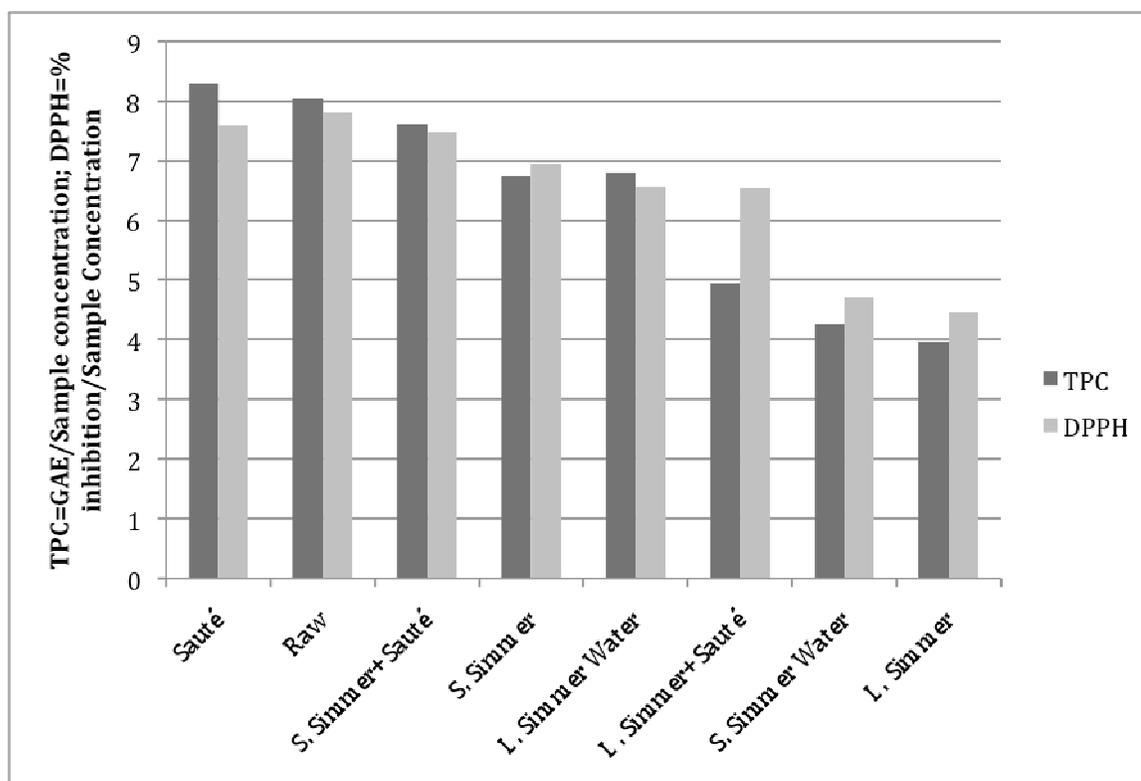
(TEAC), Ferric Reducing Ability of Plasma (FRAP) and Oxygen Radical Absorbance Capacity (ORAC) assays (Proteggente et al., 2002) and in fruits with DPPH, FRAP, ORAC and 2,2-azinobis(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) assays (Thaipong et al. 2006).

In testing the effects of thermal treatments on the content of various polyphenols in artichoke, Ferracane et al (2008) reported that most thermal treatments increased the bioavailability of polyphenols most likely as a result of a weakening of the plant biomass. A similar mechanism could also account for the increase in total phenolic content found in sauté collard greens when compared to raw greens in the current study. When an aquathermal treatment was applied, measures for both TPC and DPPH decreased with longer simmering times. Aquathermal treatment of kale, another Brassica variant, also caused reductions in total phenolics and radical scavenging activity (Sikora et al. 2008).

The order trend of the FIC assay was noticeably different from both the TPC and DPPH assays (Figure 5). Both of the Simmer water treatments showed the lowest ferrous ion chelating ability of any of the treatments. Sauté and Raw groups, which were the two strongest groups in the other two assays were 4<sup>th</sup> and 3<sup>rd</sup> weakest respectively in the FIC assay. The Folin-Ciocalteu reagent used in the TPC assay has been found to react with chelating molecules (Prior et al., 2005) yet in various experiments there was found to be no strong correlation between TPC and FIC assay results (Chew et al., 2008; Gulcin et al., 2008; Sun

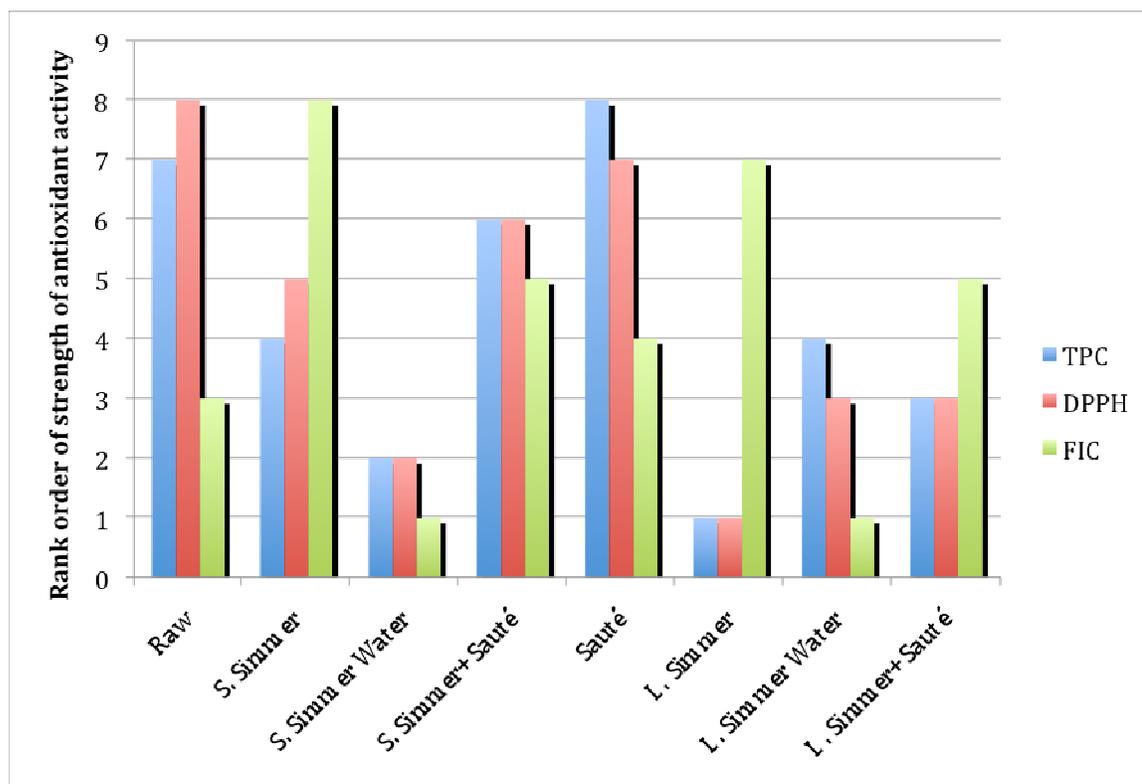
et al., 2009). As opposed to the DPPH and TPC assays where antioxidant activity and phenolics were lost to the simmer water during aquathermal treatment, similar leeching was not observed with metal chelating compounds found in collard greens. Length of aquathermal treatment did not affect the chelating ability of the simmer water either. Based on the current study, simmer treatments were the most optimal at increasing the activity of metal chelators. This increase is possibly due to degradation of biomass since the sauté treatment also increased the metal chelating activity compared to other treatments.

Figure 4. Results for TPC and DPPH assays.



TPC and DPPH antioxidant assay results. TPC results are expressed in Gallic acid equivalents/mg/ml. DPPH results are expressed in % change in absorbance/Sample concentration.

Figure 5. Relation of relative ranks of assay results for all assays.



Antioxidant assay results ranked in order of lowest activity (1) to highest activity (8). No significant difference between groups of same color and rank ( $p \leq 0.05$ ).

### Conclusion

Primary antioxidants as measured in the electron transfer based DPPH and Folin-Ciocalteu assays were negatively impacted by aquathermal treatment. Total antioxidant measures decreased from an increase in treatment duration and more antioxidant activity was found in the wastewater of the long simmer treatments compared to the short simmer treatments. Both antioxidant capacity and total phenolics were found to increase when aquathermal treatments were subjected to additional sauté treatment with deionized water. Further increases

could possibly be measured by using the wastewater of a simmer method as the liquid for the sauté or even with using a regular vegetable oil as in a traditional sauté method. Kalogeropoulos et al. (2007) found that they were able to transfer a significant amount of polyphenols from olive oil into fish fried in the olive oil. However because this supplementation would provide lipophilic polyphenols, it would not be a direct replacement for the most likely hydrophilic polyphenols that are lost during aquathermal treatment.

While DPPH and TPC assays followed similar ranking order, they varied on which group was the strongest. For the DPPH assay, the raw group was significantly higher than the sauté group while in the TPC assay the sauté group actually showed greater values than the untreated raw group. Such an increase in total phenolics could be explained by the thermal treatment's ability to break down the biomass of the collard greens allowing for greater availability of polyphenols contained inside the cell walls (Ferracane et al., 2008).

Following a different ranking trend than seen in DPPH and TPC assays, the FIC assay found greater chelating ability in the samples that had undergone aquathermal treatment compared to raw and sauté groups. Additionally very low values of chelating ability were found in the reserved wastewater samples for both the long and short simmer treatments that were not significantly different from each other. The low chelating content of the reserved water reinforces the

need for more than just water as a solvent when testing for chelating compounds in collard greens.

Further experimentation would be useful not only in determining if the above assumption of antioxidant retention through using simmer wastewater as a liquid in sautéing is correct but also as to how such a use would affect overall taste acceptance of the resulting product. While the combined values of the simmer and reserved water groups exceeded that of the other groups antioxidant assays were not performed specifically on combined samples such as this. Because of the lack of actual testing on combined simmer+reserved water groups it is not possible to reliably compare results to the measured treatments. Traditional comfort food cooking of collard greens often times uses the wastewater as a flavoring liquid in the final dish, referring to it as the “pot liquor”. As the results of this experiment show, this pot liquor has significant amounts of antioxidants making it quite beneficial to consume. Additionally polyphenolic content is not the only thing lost during aquathermal treatment. In a similar experiment with kale, it was found that aquathermal treatment caused a significant decrease in the amino acid content of the samples (Lisiewska et al. 2008) and so follow-up experiments would be useful in determining the effect of cooking techniques used in this study on more than just primary and secondary antioxidant measures of collard greens.

Through the use of three different assays that measure various aspects of antioxidants it was found that aquathermal treatments had a significant affect. In the cases where hydrophilic compounds appeared to constitute a considerable portion of total antioxidant power aquathermal treatments such as a short term simmer and a long term simmer were shown to decrease the antioxidant measures. Through the use of an additional cooking treatment of sauté both long term and short term simmer treatment measures were found to increase. As such this study supports the use of a two-step cooking method for collard greens when aquathermal treatment is used. The importance of this two-step method appears to be restricted to hydrophilic antioxidants.

### **References**

- Ardestani, A, Yazdanparast, R. 2007. Antioxidant and Free Radical Scavenging Potential of Achillea santolina Extracts. Food Chemistry. 104: 21-29.
- Bazzano, LA, He, J, Ogden, LG, Loria, CM, Vupputuri, S, Myers, L, Whelton, PK. 2002. Fruit and Vegetable Intake and Risk of Cardiovascular Disease in US Adults: the First National Health and Nutrition Examination Survey Epidemiologic Follow-Up Study. 76(1): 93-99.
- Chew, YL, Lim, YY, Omar, M, Khoo, KS. 2008. Antioxidant Activity of Three Edible Seaweeds from Two Areas in South East Asia. Food Science and Technology. 41: 1067-1072.
- The Culinary Institute of America. 2000. The Professional Chef's Techniques of Healthy Cooking. New York, NY: John Wiley & Sons.
- Ferracane, R, Pellegrini, N, Visconti, A, Graziani, G, Chiavaro, E, Miglio, C, Fogliano, V. 2008. Effects of Different Cooking Methods on Antioxidant Profile, Antioxidant Capacity, and Physical Characteristics of Artichoke. Journal of Agricultural and Food Chemistry. 56: 8601-8608.

- Gazzani, G, Papetti, A, Massolini, G, Daglia, M. 1998. Anti- and Prooxidant Activity of Water Soluble Components of Some Common Diet Vegetables and the Effect of Thermal Treatment. *Journal of Agriculture and Food Chemistry*. 46: 4118-4122.
- Gulcin, I, Tel, AZ, Kirecci, E. 2008. Antioxidant, Antimicrobial, Antifungal, and Antiradical Activities of *Cyclotrichium niveum* (boiss.) Manden and Scheng. *International Journal of Food Properties*. 11: 450-471.
- Kalogeropoulos, N, Chiou, A, Mylona, A, Ioannou, MS, Andrikopoulos, NK. 2007. Recovery and Distribution of Natural Antioxidants (alpha-Tocopherol, Polyphenols and Terpenic Acids) After pan-Frying of Mediterranean Finfish in Virgin Olive Oil. *Food Chemistry*. 100: 509-517.
- Lim, YY, Lim, TT, Tee, JJ. 2007. Antioxidant Properties of Several Tropical Fruits: A Comparative Study. *Food Chemistry*. 103: 1003-1008.
- Lisiewska, Z, Kmiecik, W, Korus, A. 2008. The Amino Acid Composition of Kale (*Brassica oleracea* L. var. *acephala*), Fresh and After Culinary and Technological Processing. *Food Chemistry*. 108: 642-648.
- Lisiewska, Z, Kmiecik, W, Korus, A. 2008. The Amino Acid Composition of Kale (*Brassica oleracea* L. var. *acephala*), Fresh and After Culinary and Technological Processing. *Food Chemistry*. 108: 642-648.
- McGee, H. 1984. *On Food and Cooking*. New York, NY: Scribner.
- Moure, A, Cruz, JM, Franco, D, Dominguez, JM, Sineiro, J, Dominguez, H, Nunez, MJ, Parajo, JC. 2001. Natural Antioxidants from Residual Sources. *Food Chemistry*. 72: 145-171.
- Prior, RL, Wu, X, Schaich, K. 2005. Standardized Methods for the Determination of Antioxidant Capacity and Phenolics in Foods and Dietary Supplements. *Journal of Agricultural and Food Chemistry*. 53 (10): 4290-4302.
- Prior, RL, Wu, X, Schaich, K. 2005. Standardized Methods for the Determination of Antioxidant Capacity and Phenolics in Foods and Dietary Supplements. *Journal of Agricultural and Food Chemistry*. 53 (10): 4290-4302.
- Proteggente, AR, Pannala, AS, Paganga, G, Van Buren, L, Wagner, E, Wiseman, S, Van de Put, F, Dacombe, C, Rice-Evans, CA. 2002. The Antioxidant Activity of Regularly Consumed Fruit and Vegetables Reflects their Phenolic and Vitamin C Composition. *Free Radicals Research*. 36 (2): 217-233.
- Roy, MK, Takenaka, M, Isobe, S, Tsushida, T. 2007. Antioxidant Potential, Anti-Proliferative Activities, and Phenolic Content in Water-Soluble Fractions of Some

Commonly Consumed Vegetables: Effects of Thermal Treatment. *Food Chemistry*. 103: 106-114.

Roy, MK, Takenaka, M, Isobe, S, Tsushida, T. 2007. Antioxidant Potential, Anti-Proliferative Activities, and Phenolic Content in Water-Soluble Fractions of Some Commonly Consumed Vegetables: Effects of Thermal Treatment. *Food Chemistry*. 103: 106-114.

Sikora, E, Cieslik, E, Leszczynska, T, Filipiak-Florkiewicz, A, Pisulewski, PM. 2008. The Antioxidant Activity of Selected Cruciferous Vegetables Subjected to Aquathermal Processing. *Food Chemistry*. 107: 55-59.

Singh, N, Rajini, PS. 2004. Free Radical Scavenging Activity of an Aqueous Extract of Potato Peel. *Food Chemistry*. 85: 611-616.

Singleton, VL, Orthofer, R, Lamuela-Raventos, RM. 1999. Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. *Methods in Enzymology*. 299: 152-178.

Smith, SA, King, RE, Min, DB. 2007. Oxidative and Thermal Stabilities of Genetically Modified High Oleic Sunflower Oil. *Food Chemistry*. 102: 1208-1213.

Sun, YP, Chou, CC, Yu, RC. 2009. Antioxidant Activity of Lactic-Fermented Chinese Cabbage. *Food Chemistry*. 115: 912-917.

Thaipong, K, Boonprakob, U, Crosby, K, Cisneros-Zevallos, Luis, Byrne, DH. 2006. Comparison of ABTS, DPPH, FRAP, and ORAC Assays for Estimating Antioxidant Activity of Guava Fruit Extracts. *Journal of Food Composition and Analysis*. 19: 669-675.

Volden, J, Bengtsson, GB, Wicklund, T. 2009. Glucosinolates, l-Ascorbic Acid, Total Phenols, Antocyanins, Antioxidant Capacities and Colour in Cauliflower (*Brassica oleracea* L. ssp. *Botrytis*); Effects of Long-Term Freezer Storage. *Food Chemistry*. 112: 967-876.

Volden, J, Borge, GIA, Bengtsson, GB, Hanse, M, Thygesen, IE, Wicklund, T. 2008. Effect of Thermal Treatment on Glucosinolates and Antioxidant-Related Parameters in Red Cabbage (*Brassica oleracea* L. ssp. *Capitata* f. *rubra*). *Food Chemistry*. 109: 595-605.

Wachtel-Galor, S, Wong, KW, Benzie, IFF. 2008. The Effect of Cooking on Brassica Vegetables. *Food Chemistry*. 110: 706-710.

Xu, B, Chang, SKC. 2008. Effect of Soaking, Boiling, and Steaming on Total Phenolic Content and Antioxidant Activities of Cool Season Food Legumes. *Food Chemistry*. 110: 1-13.