

Culinary Method Affects the Antioxidant Activity of Collard Greens (*Brassica oleracea*)

Alexander Clifford¹ & Paul Dawson¹

¹Department of Food, Nutrition and Packaging Sciences, Clemson University, Clemson, SC, USA

Correspondence: Paul Dawson, Department of Food, Nutrition and Packaging Sciences, Clemson University, Clemson, SC 29634, USA. Tel: 864-656-1138. E-mail: pdawson@clemson.edu

Received: September 9, 2012 Accepted: September 19, 2012 Online Published: October 25, 2012

doi:10.5539/jfr.v1n4p66

URL: <http://dx.doi.org/10.5539/jfr.v1n4p66>

Abstract

The antioxidant activity of collard greens was determined after exposed to eight different thermal treatments: 1) untreated raw group, 2) short simmer 3) short simmer water 4) short simmer + sauté, 5) sauté 6) long simmer 7) long simmer water 8) long simmer + sauté. After treatment, 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 determined. 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 were not relatively water soluble and therefore not negatively affected by aquathermal treatments.

Keywords: antioxidant activity, total phenolic content, collard greens, free radical scavenging activity, iron chelating

1. Introduction

1.1 Antioxidants

Antioxidants continue to be a focus in food 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, and 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, Wu, & Schaich, 2005).

1.2 Cooking and 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, Nelson, Kilgore, & Trevathan, 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, Janitha, & Wanasundara, 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, Riccio, Bergero, Del Re, & Trevisan, 2007).

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, King and Min, 2007; Singh & Rajini, 2004) and has been well documented in different varieties of the *Brassica oleracea* species (Gazzani, Papetti, Massolini, & Daglia, 1998; Lisiewska, Kmecik, & Korus, 2008; Volden et al., 2008; Roy, Takenka, Isobe, & Tsushida, 2007; Wachtel-Galor, Wong, & Benzie, 2008).

1.3 Hypothesis

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). Thus, the objective of this study was to determine how commonly used cooking treatments affect the antioxidant content of collard greens (*Brassica oleracea*).

2. Methods

2.1 Reagents, Instrumentation and Raw Materials

Reagents used for the various tests were 3,4,5-trihydroxybenzoic acid (gallic acid (Sigma-Aldrich, St. Louis, MO 63103)), sodium bicarbonate (Sigma-Aldrich), 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma-Aldrich), 5,6-diphenyl-1,2,4-ferrozine (Sigma-Aldrich), FeCl₂ 98% (ferrous chloride (Sigma-Aldrich), Folin-Cocalteau Reagent (2N FCR (Sigma-Aldrich)) and Ethyl Alcohol 200 Proof (Deacon Laboratories Inc. UK) Distilled water was obtained from a Nanopure Infinity Ultrapure Water System unit (Thermolyne Fischer 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, Columbus, OH). Precision volumetric measurements were done using either a 1-5ml FisherbrandFinnpipette adjustable pipette (Fisher Scientific) or a 1000 microliter reference adjustable pipette (Eppendorf, Inc., New York, NY). 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. 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.

2.2 Treatments

The 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: (1 Raw; (2 Short Simmer (heated 5 min @ 82 C in 250 ml, drained); (3 Short simmer water (water drained from short simmer); (4 Short simmer+saute' (short simmered then sauted for 4 min @ 82C); (5 Saute' (heated 4 min @ 82C in saucepan); (6 Long simmer (heated 20 min @ 82C in 250 ml, drained); (7 Long simmer water (water drained from long simmer); (8 Long simmer+saute' (long simmered then sauted for 4 min @ 82C)

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° ± 2°C. The remaining treatments were as follows: Short simmer; samples were added to 250 ml 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 bagging 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.

2.3 Sample Extraction

Extraction procedures were based on Lim, Lim, and Tee (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 .

2.4 Solids Determination

The oven drying method was used to determine solids content. Approximately 2-3 g 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:

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

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.

2.5 DPPH Assay

DPPH assay procedures were based on (Ardestani & Yazdanparast, 2007). For each reading, 150 μl of sample were added to 850 μl of 50% (v/v) ethanol. To this 1 ml of 0.2 mM 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 517 nm. 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:

$$\text{Free radical scavenging activity (\%/mg/ml)} = [(A_0 - A_1) / A_0] \times 100\% / \text{Conc}_1$$

where A_0 is the absorbance of the control, A_1 is the absorbance of sample and Conc_1 is the mg/ml concentration of the sample.

2.6 Total Phenolics

Total phenolic content was measured according to the Folin-Ciocalteu reaction (Singleton, Orthofer, & Lamuela-Ravnetos, 1999). Briefly, 200 μl 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% NaHCO_3 was added then vortexed for 5 seconds and allowed to react for 15 minutes before absorbance was read at 765 nm. 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:

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

where A_1 is the absorbance of the sample and $Conc_1$ is the concentration of sample.

2.7 Ferrous Ion Chelating

Chelating ability of the samples was determined by the ferrous ion chelating assay (Gulcin et al., 2008). From each of the sample extracts, 0.4 ml was taken and mixed with 0.2ml of 2mM $FeCl_2$. 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 ($\sim 24^\circ C$) 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- Fe^{2+} complex was reported in relation to samples dry mass by using the formula:

$$\text{Ferrous ion chelating activity (\%/mg/ml)} = [(A_0 - A_1)/A_0] \times 100\% / Conc_1$$

where A_0 is the absorbance of the control, A_1 is the absorbance of sample and $Conc_1$ is the mg/ml concentration of the sample.

2.8 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.

3. Results

3.1 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 (Table 1). The raw (R) followed by the short simmer + sauté (SS+S) treatments contained the next highest TPC. The long simmer water (LSW) was not different from the short simmer group (SS) ($p=0.246$). Gallic acid equivalents descended through the remaining treatments of long simmer + sauté (LS+S), short simmer water (SSW) and long simmer (LS). 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.

Table 1. Total phenolic content (TPC), 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and ferrous chelating capacity (FIC) of collard greens prepared using different cooking methods

	TPC	DPPH	FIC
Raw	8.03b	7.79a	4.12e
Short Simmer (heated 5 min @ 82 C in 250 ml, drained)	6.73d	6.94d	5.84a
Short simmer water (water drained from short simmer)	4.27f	4.70f	3.54f
Short simmer+saute' (short simmered then sauted for 4 min @ 82C)	7.62c	7.47c	4.87c
Saute' (heated 4 min @ 82C in saucepan)	8.28a	7.58b	4.69d
Long simmer (heated 20 min @ 82C in 250 ml, drained)	3.95g	4.46g	5.43b
Long simmer water (water drained from long simmer)	6.79d	6.56e	3.58f
Long simmer+saute' (long simmered then sauted for 4 min a@82C)	4.95e	6.63e	4.97c
Standard error of the mean	0.11	0.13	0.13

a-g means with different letter 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 (517 nm)/mg/ml).

FIC = ferrous iron chelating capacity (% change in absorbance (562 nm) /mg/ml).

3.2 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 R, S, SS+S, SS, LSW and LS+S, SSW and lastly LS (Table 1). Treatments not differing in DPPH activity were the LSW and LS+S groups ($p=0.590$). All other groups were significantly different at ($p\leq 0.05$).

3.3 Ferrous Iron Chelating

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 Fe^{2+} was the SS (Table 1). The next different treatment was the LS. The LS+S and the SS+S did not differ ($p=0.083$) in chelating capacity. The fourth and fifth best chelating treatments were the S and R groups, respectively. The remaining two treatments of LSW and SSW had the lowest chelating capacity and did not significantly differ from each other ($p=0.409$).

4. Discussion

The order of antioxidant strength of cooking treatments for both the total phenolics and DPPH assays followed a similar trend (Figure 1). In both cases the weakest group was LS, followed by SSW and then LS+S. In both assays the two strongest groups were S and R and the third strongest treatment in both assays was Short simmer + sauté. Other researchers have found similar relationships between TPC and DPPH assays (Chew, Lim, Omar, & Khoo, 2008; Sikora, Cieslik, Leszczynska, Filipiak-Florkiewicz, & Pisulewski, 2008; Xu & 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-azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) assays (Thaipong, Boonprakob, Crosby, Cisneros-Zevallos, & Byrne, 2006).

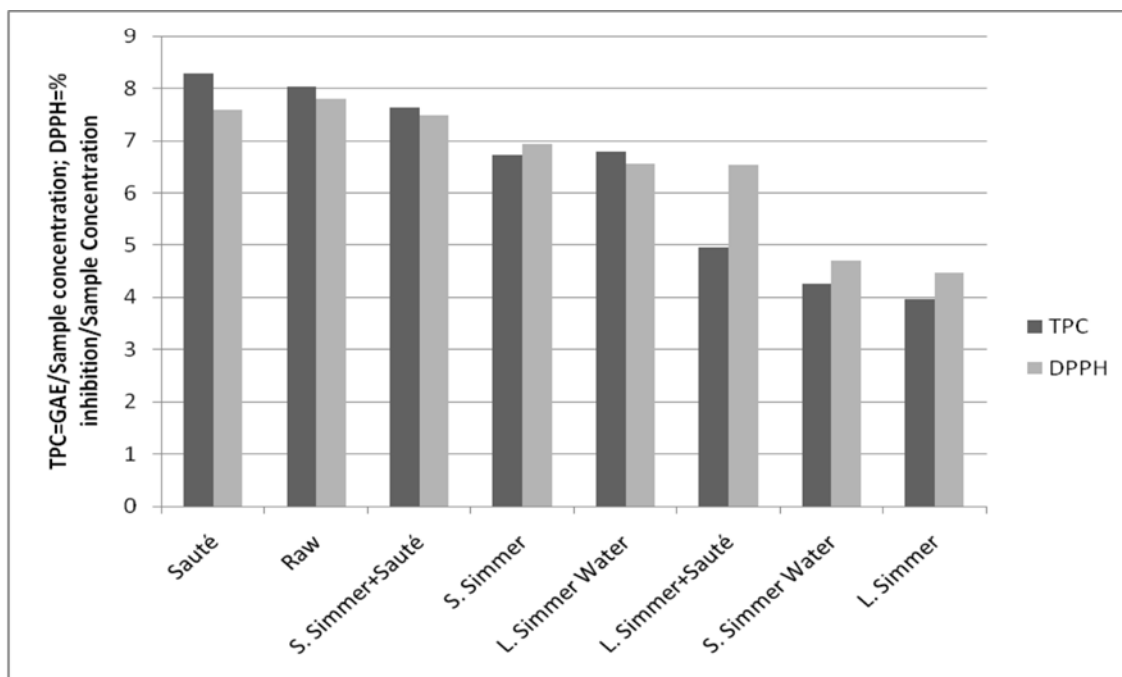


Figure 1. Total phenolic content (TPC) and DPPH activity for collard greens prepared using different cooking methods. Standard error of the mean for TPC = 0.11, standard error of the mean for DPPH = 0.13

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 2). Both of the simmer water treatments (SSW and LSW) 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 4th and 3rd 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 no strong correlation between TPC and FIC assay results (Chew et al., 2008; Gulcin & Kirecci, 2008; Sun, Chou, & Yu, 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. Metal chelating substances found in plant material include chlorophyll, phytic acid, phospholipids, oxalates and possibly carotenoids. Since length of aquathermal treatment did not affect the chelating ability of the simmer water the chelating compounds in collard greens may be predominately lipid soluble. 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.

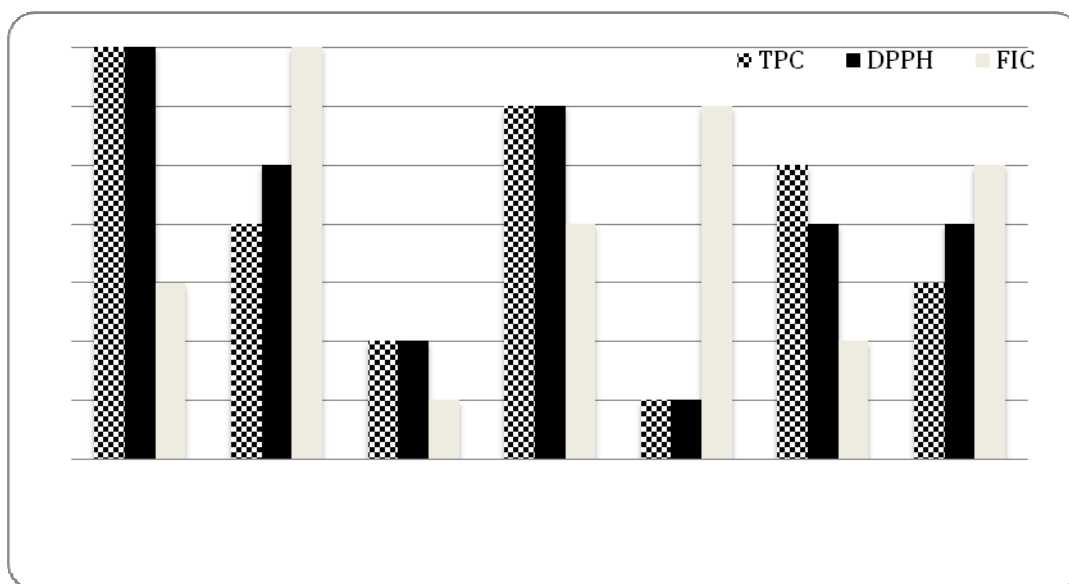


Figure 2. Relative rank of each antioxidant assay for each cooking method. Each antioxidant assay was ranked from lowest (1) to highest (8) for each cooking method

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, Chiou, Mylona, Ioannou, and Andikopoulos (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 it was determined that aquathermal treatments had a significant effect on the various antioxidants modes of activity. 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 activity. Through the use of an additional cooking treatment of sauté both long term and short term simmer treatments increased antioxidant activity. As such this study supports the use of a two-step cooking method for collard greens to maximize antioxidant activity when water cooking is used. The importance of this two-step method is restricted to hydrophilic antioxidants.

References

- Ardestani, A., & Yazdanparast, R. (2007). Antioxidant and free radical scavenging potential of *Achilleasantolina* extracts. *Food Chemistry*, *104*, 21-29. <http://dx.doi.org/10.1016/j.foodchem.2006.10.066>
- Bazzano, L. A., He, J., Ogden, L. G., Loria, C. M., ORIA, Vupputuri, S., Myers, L., & Whelton, P. K. (2002). Fruit and vegetable intake and risk of cardiovascular disease in US adults. *First National Health and Nutrition Examination Survey Epidemiologic Follow-Up Study*, *76*(1), 93-99.
- Chew, Y. L., Lim, Y. Y., Omar, M., & Khoo, K. S. (2008). Antioxidant activity of three edible seaweeds from two areas in South East Asia. *Food Science and Technology*, *41*, 1067-1072.
- Ferracane, R., Pellegrini, N., Visconti, A., Graziani, G., Chiavaro, E., Milglio, C., & Foglinano, 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. <http://dx.doi.org/10.1021/jf800408w>
- 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. <http://dx.doi.org/10.1021/jf980300o>
- Greco, L., Riccio, L., Bergero, S., Del Re, A. M. M., & Trevisan, M. (2007). Total reducing capacity of fresh sweet peppers and five different Italian pepper recipes. *Food Chemistry*, *103*, 1127-1133. <http://dx.doi.org/10.1016/j.foodchem.2006.10.013>
- Gulcin, I., Tel, A. Z., & Kirecci, E., (2008). Antioxidant, antimicrobial, antifungal, and antiradical activities of *Cyclotrichiumniveum* (boiss.) manden and scheng. *International Journal of Food Properties*, *11*, 450-471. <http://dx.doi.org/10.1080/10942910701567364>
- Jurman, R., Nelson, H., Kilgore, L., & Trevathan. (2005). *Introduction to Physical Anthropology 8th Edition*. Belmont, CA: Wadsworth.
- Kalogeropoulos, N., Chiou, A., Mylona, A., Ioannou, M. S., & Andrikopoulos, N. K. (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.

- <http://dx.doi.org/10.1016/j.foodchem.2005.09.072>
- Lim, Y. Y., Lim, T. T., & Tee, J. J. (2007). Antioxidant properties of several tropical fruits: A comparative study. *Food Chemistry*, *103*, 1003-1008. <http://dx.doi.org/10.1016/j.foodchem.2006.08.038>
- Lisiewska, Z., Kmecik, 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. <http://dx.doi.org/10.1016/j.foodchem.2007.11.030>
- McGee, H. (1984). *On Food and Cooking*. New York, NY: Scribner.
- Moure, A., Cruz, J. M., Franco, D., Dominguez, J. M., Sinerio, J., Dominguez, H., ... Parajo, J. C. (2001). Natural antioxidants from residual sources. *Food Chemistry*, *72*, 145-171. [http://dx.doi.org/10.1016/S0308-8146\(00\)00223-5](http://dx.doi.org/10.1016/S0308-8146(00)00223-5)
- Prior, R. L., 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. <http://dx.doi.org/10.1021/jf0502698>
- Proteggente, A. R., Pannala, A. S., Paganga, G., Van Buren, L., Wagner, E., Van De Put, F., ... Rice-Evans, C. A. (2002). The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition. *Free Radicals Research*, *36*(2), 217-233. <http://dx.doi.org/10.1080/10715760290006484>
- Roy, M. K., Takenka, 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. <http://dx.doi.org/10.1016/j.foodchem.2006.08.002>
- Shahidi, F., Janitha, P. K., & Wanasundara, P. D. (1992). Phenolic antioxidants. *Critical Reviews in Food Science and Nutrition*, *32*(1), 67-103. <http://dx.doi.org/10.1080/10408399209527581>
- Sikora, E., Cieslik, E., Leszczynska, T., Filipiak-Florkiewicz, A., & Pisulewski, P. M. (2008). The antioxidant activity of selected cruciferous vegetables subjected to aquathermal processing. *Food Chemistry*, *107*, 55-59. <http://dx.doi.org/10.1016/j.foodchem.2007.07.023>
- Singh, N., & Rojimi, P. S. (2004). Free radical scavenging activity of an aqueous extract of potato peel. *Food Chemistry*, *85*, 611-616. <http://dx.doi.org/10.1016/j.foodchem.2003.07.003>
- Singleton, V. L., Orthofer, R., & Lamuela-Ravnetos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, *299*, 152-178. [http://dx.doi.org/10.1016/S0076-6879\(99\)99017-1](http://dx.doi.org/10.1016/S0076-6879(99)99017-1)
- Smith, S. A., King, R. E., & Min, D. B. (2007). Oxidative and thermal stabilities of genetically modified high oleic sunflower oil. *Food Chemistry*, *102*, 1208-1213. <http://dx.doi.org/10.1016/j.foodchem.2006.06.058>
- Sun, Y. P., Chou, C. C., & Yu, R. C. (2009). Antioxidant activity of lactic-fermented Chinese cabbage. *Food Chemistry*, *115*, 912-917. <http://dx.doi.org/10.1016/j.foodchem.2008.12.097>
- Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., & Byrne, D. H. (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. <http://dx.doi.org/10.1016/j.jfca.2006.01.003>
- The Culinary Institute of America. (2000). *The Professional Chef's Techniques of Healthy Cooking*. New York, NY: John Wiley & Sons.
- Volden, J., Bengtsson, G. B., & Wicklund, T. (2009). Glucosinolates, l-ascorbic acid, total phenols, anthocyanin's, antioxidant capacities and colour in cauliflower (*Brassica oleracea* L. ssp. *botrytis*); effects of long-term freezer storage. *Food Chemistry*, *112*, 967-976. <http://dx.doi.org/10.1016/j.foodchem.2008.07.018>
- Volden, J., Borge, G. I. A., Bengtsson, G. B., Hanse, M., Thygesen, I. E., & 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. <http://dx.doi.org/10.1016/j.foodchem.2008.01.010>
- Wachtel-Galor, S., Wong, K. W., & Benzie, I. F. F. (2008). The effect of cooking on Brassica vegetables. *Food Chemistry*, *110*, 706-710. <http://dx.doi.org/10.1016/j.foodchem.2008.02.056>
- Xu, B., & Chang, S. K. C. (2008). Effect of soaking, boiling, and steaming on total phenolic content and antioxidant activities of cool season food legumes. *Food Chemistry*, *110*, 1-13. <http://dx.doi.org/10.1016/j.foodchem.2008.01.045>