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Effect of Cooking Regime on the Antioxidant Content of Cabbage, Cucumber and Spinach

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Abstract

Fruits and vegetables have been reported to be good sources of antioxidants which are very essential in the management and prevention of free radical or reactive oxygen species related illnesses. However, there are concerns on how these foods should be prepared in order to retain their nutritive value. The effect of cooking time and method on the antioxidant content of cabbage, cucumber and spinach was studied. Raw samples of cabbage, cucumber and spinach were washed and cut into pieces. The samples were then cooked by microwaving for 1, 3 and 5 minutes. They were also cooked by steaming and boiling for 2, 5 and 10 minutes. The water-soluble antioxidant content of the raw cabbage, cucumber and spinach was found to be 1.605mg/100ml, 1.161mg/100ml and 2.085mg/100ml respectively. The total phenolic content of the raw sample was found to be 1.259 mgGAE/g, 1.232mgGAE/gand 1.124mgGAE/g for cabbage, cucumber and spinach respectively while the total flavonoid content of the raw samples was found to be 0.111 mgQE/g, 0.108 mgQE/g and 0.108 mgQE/g for cabbage, cucumber and spinach respectively. Cooking method and time had effects on the antioxidant content of the samples. Microwaving and boiling both reduced antioxidant content of all samples with increase in time when compared to the unprocessed samples. Steaming, however, showed an increase in the antioxidant content of spinach and cucumber when compared to the unprocessed samples. Steaming spinach and cucumber for 2 minutes gave the highest antioxidant capacity. Highest antioxidant content of cabbage was shown by the unprocessed samples. However, steaming cabbage for 5 minutes gave the highest antioxidant capacity.This study shows that processing has an effect on the antioxidant content of these vegetables and steaming may be considered as a method of choice for processing these vegetables when antioxidant property is in view.

Keywords: Antioxidant, flavonoid, phenolic, microwaving, steaming and boiling

Introduction

In recent years, increasing attention has been paid by humans to the role of diet in human health. Epidemiological studies have shown that a high intake of plant-originated foods is strongly associated with a reduced risk of a number of chronic diseases, such as cancer and atherosclerosis,[18] neurodegenerative diseases, including Parkinson's and Alzheimer's diseases as well as inflammation, problems caused by cell, cutaneous aging[6] cataract, macular degeneration[23] and diabetes[2] These beneficial effects have been partly attributed to the compounds present in plants that possess antioxidant content. Also, antioxidant compounds possess antimutagenic, antibacterial, or antiviral activities to a greater or lesser extent. According to cai,[2]antioxidant compounds, found in plants (fruits, vegetables, medicinal herbs, etc.), include free radical scavenging molecules, such as phenolic compounds (e.g., phenolic acids, flavonoids, quinones, stilbenes, lignans, coumarins, tannins), nitrogen compounds (alkaloids, amines, betalains), endogenous metabolites,vitamin (C, E), and terpenoids (including carotenoids).Among horticultural crops, fruits are most commonly consumed raw; however, Vegetables are generally cooked by different cooking processes, including boiling in water or microwaving, before being consumed. These processes alter the physical and chemical properties of vegetables.[23] Cooking processes may have an effect on the antioxidant content of food due to antioxidant release, destruction, or creation of redox active metabolites.[7] Wachtel-Galor,[24] reported that antioxidant compounds, such as ascorbic acid and some carotenoids, are very sensitive to heat and storage. Conversely, polyphenols have shown certain stability when exposed to high temperatures. According to Cai[7] previous studies conducted on different vegetables showed that, after cooking, total polyphenol content and antioxidant content of samples were higher or lower compared to fresh vegetables. They reported that cooking decreased antioxidant content for most of the vegetables and that small differences were found between the cooking methods applied. Wachtel-Galor[24] reported that antioxidant content was lowest in microwaved samples and was followed by boiled and steamed samples and decreased with longer cooking time, regardless of the method in all cooked vegetables. Antioxidant contents increased for all steamed vegetables over that of raw vegetables. Effects were variable for boiling and microwaving. Boiling caused lesser antioxidant loss in cooking than did microwaving. Turkmen[23] reported that after cooking, total antioxidant content increased or did not change depending on the type of vegetable but not type of cooking. Zhang and Hamauzu pointed out that antioxidant components and antioxidant content in broccoli samples were lost quickly during cooking.

Vegetables would either be eaten uncooked or more commonly eaten after cooking by steaming, boiling, or microwaving. Cooking methods have major importance on nutritional values of vegetables.

Phenolic compounds comprise a wide variety of molecules that have a polyphenol structure. Polyphenols are divided into several classes according to the number of phenol rings that they contain and to the structural elements that bind these rings to one another. The main groups of polyphenols are: flavonoids, phenolic acids, tannins (hydrolysable and condensed), stilbenes and lignans.[12]

More than 8000 polyphenolics, including over 4000 flavonoids have been identified, and the number is still growing.[8] Flavonoids can be further classified into anthocyanins, flavones, isoflavones, flavanones, flavonols and flavanols.[23]

Flavonoids are low molecular weight compounds, consisting of fifteen carbon atoms, arranged in a C6–C3–C6 configuration.[16] Flavonoids are especially important antioxidants due to their high redox potential, which allows them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. In addition, they have a metal chelating potential.[22] Flavonoids are the most commonly found phytochemicals, that typically help to protect the plant against UV light, fungal parasites, herbivores, pathogens and oxidative cell injury. When consumed regularly by humans, flavonoids have been associated with a reduction in the incidence of diseases such as prostate.[10,20]

Cucumber (*Cucumis sativus*) has numerous wild African species, and it has therefore been assumed that melon originated in Africa. The African group (melon group) has 30 species divided into six subgroups.[13] This crop has a special feature that the fruits can be stored up to 8-10 months without losing their freshness. The Fruits can be stored for many weeks by hanging them from the ceiling, firmly bound by thin coconut fibre ropes (Vidya, 2012). Cucumbers, which are related to melons, such as watermelon, cantaloupe and honeydew, are a relatively low-calorie food at just about 15 calories per cup, and are about 95% water.[9] They contain high levels of lignans, vitamin K, cucurbitacins and their derivatives (triterpenoids), flavonoids (apigenin, luteolin, quercetin, and kaempferol), antioxidants such as beta carotene and vitamin C, and B vitamins, among other trace elements and minerals.[17]

Cabbage (Brassica *oleracea L. var. capitata*) is one of the most important vegetables grown worldwide. It belongs to the family Cruciferae, which includes broccoli, cauliflower, and kale. The different cultivated types of cabbage show great variation in respect of size,

shape and colour of leaves as well as the texture of the head[21] Approximately 6.3 kg of Brassica vegetables are consumed per person annually.[11] Cabbage is consumed either raw or processed in different ways, e.g., boiled or, fermented or, used in salads. Cabbages have proven to be beneficial for health by numerous epidemiological and clinical studies.[18,4] High intake of cabbages for consumers could reduce the risk of degenerative diseases, age-related chronic illnesses[14] and several types of cancer.[1]

Spinach (*Spinacia oleracea*)s an annual herb belongs to the family Chenopodiaceae. It is native to Southwest Asia and widely distributed and cultivated through the world including Iran as vegetables for its high nutritious. Value.[3] Spinach is a good source of minerals (iron, copper, phosphorous, zinc, selenium), vitamin B complex (niacin and folic acid), ascorbic acid, carotenoids (ß-carotene, lutein, zeaxanthin), phenols (flavonoids, p-coumaric acid), apocynin and Omega-3-fatty acids. It is a relatively quick-growing vegetable and easy to maintain and distinguishable by its green colour as well as smooth, oblong shaped leaves that can be either crinkled or smooth. Also, the whole plant is medicinally important and are used in traditional medicine for numerous therapeutic effects because of the presence of biological tannins and phenolic active phytochemicals such as alkaloids, flavonoids, steroids, glycosides, terpenoids.[19]

This study seeks to determine the effect of cooking regime on the antioxidant content of cabbage, spinach and cucumber.

Material and Methodology

Materials, Chemical/Reagents

Spectrophotometer, water bath, centrifuge, microwave, trichloroaceticacid, magnesium carbonate, methanol, petroleum ether Sodium chloride solution, acetone, sodium carbonate, FRAP reagent (16.67 mM ferric chloride and 8.33 mM 2,4,6-tripyridyl-*s*-triazine (TPTZ) in 250 mM acetate buffer, pH 3.6), hydrochloric acid, Folin–Ciocalteureagent $NaNO_2$ solution, $AlCl_3$ solution, NaOH solution

Methods

Sample collection

Commercially available samples of fresh, cucumber, spinach and cabbage were purchased from Wukari old market Taraba state Nigeria.

Sample preparation

Each sample was washed, cut into pieces before being subjected to different cooking methods

Experimental design

Raw samples of cabbage, cucumber and spinach were washed and cut into pieces. After which they were subjected to different cooking regimes (microwaving, boiling and steaming) at different cooking times of 1 minute, 2minutes and 3 minutes for micro-wave cooking; 2, 5 and 10 minutes for boiling, and 2, 5 and 10 minutes for steaming in order to determine the effect of cooking method on the antioxidant content, phenolic and flavonoid content of the samples.

The cooked samples were subjected to extraction using Trichloroacetic acid. The extracts were then used for the determination of Total antioxidant content (TAC), Total flavonoid content and Total phenolic content.

Extract preparation for determination of antioxidant content

The extract for the determination of antioxidant content was prepared by method of ferric reducing antioxidant power (FRAP).30ml Trichloroacetic acid (5%) was added to samples, they were ground in a pestle and mortar and left to stand for 30 minutes to precipitate protein, it was then centrifuged at 4000rpm for 20 minutes to remove the pellets. The supernatant was analysed for water-soluble antioxidant content.

Determination of water-soluble antioxidant

The determination of water-soluble antioxidant was carried out using FRAP reagent. Briefly, 2.25 ml FRAP reagent (16.67 mM ferric chloride and 8.33 mM 2,4,6-tripyridyl-*s*-triazine (TPTZ) in 250 mM acetate buffer, pH 3.6) was added to a test tube at 37^0 C, 225 ml water and 75 ml of the supernatant was added and the contents mixed. After incubation for 30 min, the absorbance was measured at 593 nm. This was compared with an 5% trichloroacetic acid blank and ascorbic acid standards prepared in 5% trichloroacetic acid at concentrations up; to 0.25 mM.

Total phenolic content

The total phenolic contentof the extract was determined by the Folin–Ciocalteu method with modification. (Kaur and Kapoor ,2002). Briefly,200 µL of crude extract (1 mg/mL) were made up to 3 mL with distilled water, mixed thoroughly with 0.5 mL of Folin–Ciocalteu reagent for 3 minutes, followed by the addition of 2 mL of 20% (w/v) sodium carbonate. The mixture was allowed to stand for a further 60 minutes in the dark, and absorbance was measured at 650 nm. The total phenolic content was calculated from the calibration curve, and the results were expressed as mg of garlic acid equivalent per gram.

Total flavonoid content

The total flavonoid content of crude extract was determined by the aluminium chloride colorimetric method (Changet al., 2007). In brief, 50µL of crude extract (1 mg/mL ethanol) were made up to 1 mL with methanol, mixed with 4 mL of distilled water and then 0.3 mL of 5% $NaNO_2$ solution; 0.3 mL of 10% $AlCl_3$ solution was added after5minutes of incubation, and the mixture was allowed to stand for 6 minutes. Then, 2 mL of 1 mol /L NaOH solution were, added, and the final volume of the mixture was brought to 10 mL with double-distilled water. The mixture was allowed to stand for 15 minutes, and absorbance was measured at 510 nm. The total flavonoid content was calculated from a calibration curve, and the result was expressed as mg quercetin equivalent per gram.

Statistical Analysis

The data obtained was analysed based on Two-way analysis of variance (ANOVA) using SPSS version 20. Significant difference was determined for means at $p < 0.05$.

Results

Table 1: Effect of cooking regime and time on the antioxidant content of spinach

Cooking Method	Cooking Time(mins)	Antioxidant content AAEq(mg/100ml)
Microwaving	1	0.777±0.005
	3	0.752±0.017
	5	0.337±0.005
Steaming	2	2.296±0.006
	5	1.463±0.010
	10	0.427±0.005
Boiling	2	0.405±0.000
	5	0.716±0.021
	10	0.340±0.028
Uncooked	0	1.161±0.013

Table 2: Effect of cooking regime and time on the antioxidant content of cabbage

Cooking Method	Cooking Time (mins)	Antioxidant content AAEq(mg/100ml)
Microwaving	1	0.952±0.020
	3	1.908±0.035
	5	1.656±0.359
Steaming	2	3.649±0.001
	5	3.110±0.001
	10	2.481±0.000
Boiling	2	1.241± 0.014
	5	1.241±0.001
	10	1.401±0.001
Uncooked	0	2.085±0.000

Values are means *± standard deviation (S.D): there is a significant difference at P< 0.05 between cooking methods and time for all cooking methods and time of cooking*

Table 3: Effect of cooking regime and time on the antioxidant content of cucumber

Cooking Method	Cooking Time(mins)	Antioxidant content AAEq(mg/100ml)
Microwaving	1	1.104±0.009
	3	1.056±0.008
	5	0.820±0.015
Steaming	2	0.981±0.013
	5	1.227±0.132
	10	1.125±0.007
Boiling	2	0.839±0.016
	5	0.237±0.004
	10	0.938±0.017
Uncooked	0	1.605±0.007

Values are means± standard deviation (S.D): there is a significant difference at P< 0.05 between cooking methods and time for all cooking methods and time of cooking

Effects of cooking methods and time on antioxidant content of cabbage, cucumber and spinach.
Total phenolic content

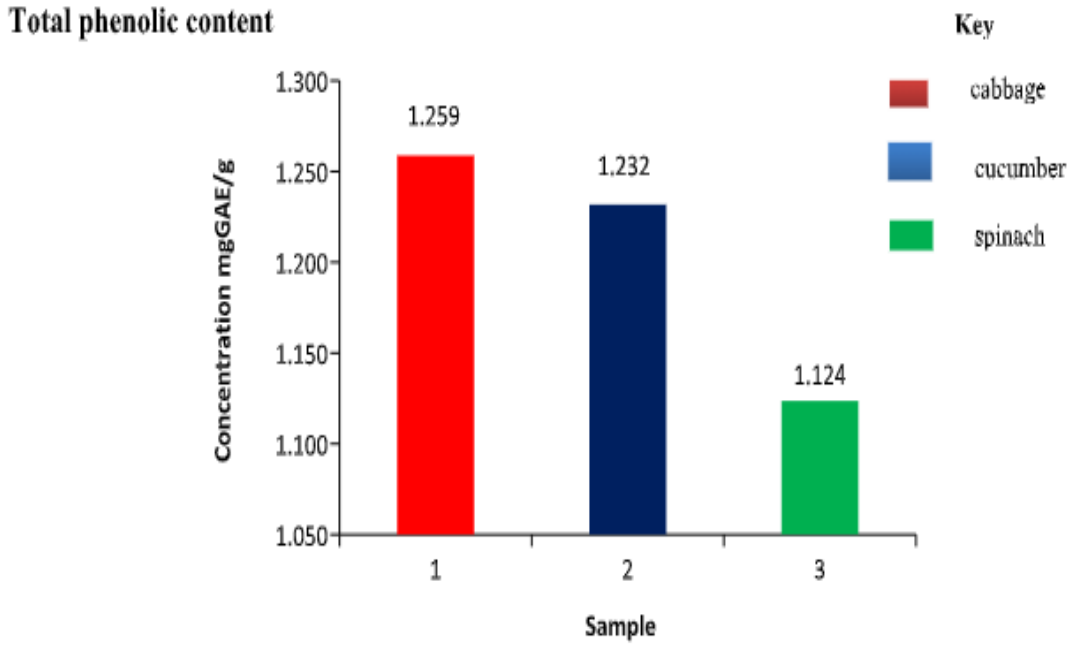

Figure 1: Total phenolic content of cabbage, cucumber.

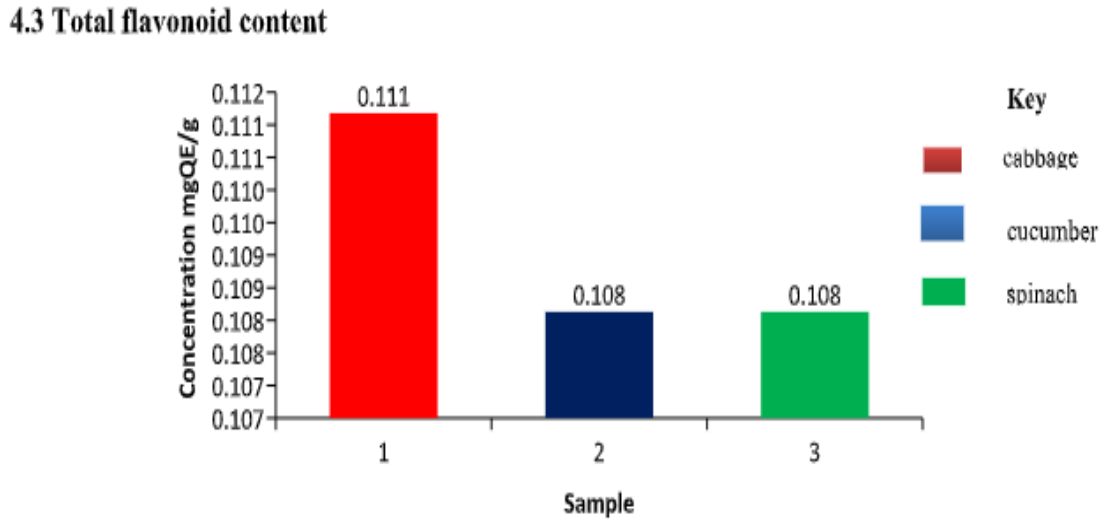

Figure 2: Total Flavonoid content of cabbage, cucumber and spinach.

Discussion

The present study considered the effect of cooking time and methods on the antioxidant capacity of selected vegetables. Raw samples of cabbage, cucumber and spinach were subjected to different cooking methods and cooking times. The samples were then extracted and the antioxidant content of the extracts were determined.

The water-soluble antioxidant content of the raw cabbage, cucumber and spinach was found to be 1.605mg/100ml, 1.161mg/100ml and 2.085mg/100ml respectively. The total phenolic content was found to be 1.259 mgGAE/g, 1.232mgGAE/g and 1.124mgGAE/g for cabbage, cucumber and spinach respectively while the total flavonoid content was found to be 0.111 mgQE/g, 0.108 mgQE/g and 0.108 mgQE/g for cabbage, cucumber and spinach respectively.

The results of the antioxidant content compares favourably with values obtained by Turkmen[23] who had 0.116mg/100ml for spinach. The difference may be due to production of redox-active secondary plant metabolites or breakdown products, but is highly likely to be related to release of antioxidants from intracellular proteins, changes in plant cell wall structure, matrix modifications, and more efficient release of antioxidants during homogenization.[24] Results for cucumber and cabbage were lower than those reported by Ismail 2004.

The presence of phenols and flavonoids supports the antioxidant content of the extracts. There was a negative correlation between the phenolic content and the antioxidant capacity. This may be due to a certain stability when exposed to high temperatures[24,7] and the method of antioxidant content determination FRAP.[15] Other methods of antioxidant content determination may be concurrently carried out to have a general perspective of the antioxidant content of the extracts. This study, however, focused on antioxidant content of the fruit/vegetable.

There was a significant decrease in the antioxidant content of cabbage, cucumber and spinach when cooked using microwave for 1minutes, 3minutes, and 5minutes. This agrees with the work of Castenmille[5] who had a significant decrease in antioxidant content of spinach using microwave for 5minutes.

The decrease may be attributed to denaturation of the antioxidants by heat.[25] The highest antioxidant content from cabbage prepared by boiling was gotten after 10minutes of boiling. Although the raw samples had a higher antioxidant capacity, boiling reduced the antioxidant content at 2 and 5minutes of boiling. The rise again after 10 minutes of boiling may be due to degradation of residues that have antioxidant content.[5,23]

Steaming produced the highest antioxidant content in all the samples. The highest antioxidant content of a heat-treated cabbage was found to be at 5minutes although there was no significant decrease after steaming for 10minutes. Spinach and cucumber had the highest antioxidant at 2 minutes of steaming. Findings from this work agree with work of[24] who reported that steaming had a better effect on the antioxidant content than steaming.

Conclusion

This study considered the effect of cooking method and time on the antioxidant content of cabbage, cucumber and spinach. Compared with most foods, vegetables contain a very high antioxidant content. This is significant in explaining the beneficial effects of vegetable consumption that has been observed in many epidemiological studies. This study shows that cooking methods and times have effects on the antioxidant content of the samples studied. Microwaving and boiling both reduced the antioxidant content of the samples when compared to the raw. Increasing the cooking time generally reduced the antioxidant content of the samples. Steaming, however, showed an increase in antioxidant content when compared to the raw samples. It could be deduced that steaming these vegetables for 2 minutes may be considered as a method of choice for processing spinach and cucumber while 5minutes of steaming cabbage may be considered if the interest is the antioxidant content of these food.

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