Full Length Research Paper

Cooking enhances the antioxidant properties of some tropical green leafy vegetables

S. A. Adefegha* and G. Oboh

Biochemistry Department, Federal University of Technology, P. M. B. 704 Akure, Nigeria.

Accepted 6 August, 2009

Most leafy vegetables undergo cooking before consumption in tropical Africa. Therefore, this study sought to evaluate the effect of cooking on the vitamin C, total phenolics, total flavonoid and antioxidant properties [1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical and 2.2- azinobis -3ethylbenzo-thiazoline- 6-sulfonate radical (ABTS*) scavenging abilities, reducing property and Fe2+ chelating ability] of some tropical green leafy vegetables; Talinium triangulare, Ocimum gratissimum, Amaranthus hybridus, Telfairia occidentalis, Ipomea batata, Cnidoscolous aconitifolius, Baselia alba and Senecio biafrae leaves. The results of the study revealed that cooking causes a significant (P < 0.05) decrease in the vitamin C [raw (321.4 - 842.0), cooked (198.2 - 638.4 mg/100 g)] content. Conversely, there was a significant (P < 0.05) increase in the total phenol [raw (146.9 - 693.8), cooked (272.9 - 1037.5 mg/100 g)], total flavonoid [raw (8.2 - 53.0), cooked (12.9 - 57.4 mg/100 g)], DPPH radical scavenging ability [raw (15.7 - 61.8), cooked (52.8 - 92.7 %)], reducing property [raw (28.3 - 61.8), cooked (43.9 - 71.6 mg/100g AAE)], Fe²⁺ chelating ability [raw (17.4 - 75.4), cooked (22.8 - 89.2%)] and ABTS* scavenging ability [raw (17.4 - 87.3), cooked (57.5 - 113.2 mmol/100 gTEAC)]. In view of this, it could be concluded that cooking decreases the vitamin C contents in all the vegetables, while it increased the phenolic content and antioxidant activities.

Key words: Vegetables, cooking, antioxidant, phenolic, vitamin C.

INTRODUCTION

Free radicals are highly reactive chemical molecules/ species such as superoxide radical, hydroxyl radical and singlet oxygen that travel around the body and cause damage to the body cells. Diseases linked to oxygen radicals and reactive oxygen species (ROS) include cancer, atherosclerosis, heart disease, stroke, diabetes mellitus, rheumatoid arthritis, osteoporosis, ulcers, sunburn, cataracts and aging (Gülçin et al., 2003). Antioxidants enzymes (made in the body) and antioxidant nutrients (found in foods) can scavenge/deactivate this reactive free radicals turning them to harmless particles (Chu et al., 2002). The most likely and practical way to fight against degenerative diseases is to improve body antioxidant status, which could be achieved by higher consumption of vegetables and fruits (Oboh and Rocha, 2007).

Antioxidants protect by contributing an electron of their own. In so doing, they neutralize free radicals and help

prevent cumulative damage to body cells and tissues (Alia et al., 2003). Much of the total antioxidant activity of fruits and vegetables is related to their phenolic content, not only to their vitamin C content (Oboh et al., 2007; Oboh and Rocha, 2008). Natural polyphenols exert their beneficial health effects by their antioxidant activity, these compounds are capable of removing free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce α -tocopherol radicals and inhibit oxidases (Amic et al., 2003)

Since many degenerative human diseases have been recognised as being a consequence of free radical damage, there have been many studies undertaken on how to delay or prevent the onset of these diseases (Sun et al., 2002). Foods from plant origin usually contain natural antioxidants that can scavenge free radicals (Sun et al., 2002; Alia et al., 2003; Zhang et al., 2001; Oboh, 2005; Oboh and Akindahunsi, 2004; Oboh and Rocha, 2007).

Green leafy vegetables are popularly used for food in many countries of the world, being a rich source of ß-carotene, ascorbic acid, minerals and dietary fiber (Sun et al., 2002; Oboh, 2005; Oboh and Akindahunsi, 2004;

^{*}Corresponding author. E-mail: fegha2000@yahoo.com.

Oboh and Rocha, 2007).

Nigeria is a tropical country with a large diversity of green leafy vegetables and most of these vegetables undergo a cooking process rather than being eaten raw. Cooking is usually carried out in order to increase the palatability and to improve the edibility of some food (Oboh and Akindahunsi, 2004). The popular thinking is that fresh fruits and vegetables are better for us than cooked ones nutrition wise. Despite this thinking, most vegetables are usually cooked (unlike fruits that are usually consumed in their raw forms) before consumption. These cooking processes could bring about a number of changes in physical characteristics and chemical composition of vegetables (Rehman et al., 2003; Zhang and Hamauzu, 2004). However, a lot of information abounds on the antioxidant capacity of tropical fruits and vegetables (Sun et al., 2002; Rehman et al., 2003; Oboh and Akindahunsi, 2004; Zhang and Hamauzu, 2004: Oboh. 2005), but there are dearth of information on the effects of cooking on ascorbic acid, total phenolics and antioxidant activities of some tropical green leafy vegetables. Therefore this study sought to evaluate the effect of cooking on the ascorbic acid, total phenolics and antioxidant activities of some tropical green leafy vegetables of medicinal value: Talinium triangulare (water leaf), Senecio biafrae (Sierra Leone bologni), Amaranthus hybridus (green amaranth), Ocimum gratissimum (wild basil), Ipomea batata (Sweet potato leaf) Telfairia occidentalis (fluted pumpkin), Baselia alba Cnidoscolus aconitifolius (Chaya) leaves.

MATERIALS AND METHODS

Materials

Fresh samples of *T. triangulare* (water leaf), *A. hybridus* (green amaranth), *O. gratissimum* (wild basil), *I. batata* (Sweet potato), *T. occidentalis* (fluted pumpkin), *C. aconitifolius* (Chaya), *B. alba* and *S. biafrae* (Sierra Leone bologni) leaves were purchased in a local market, in Akure metropolis, Nigeria. Authentication of the leaves was carried out in the Department of Biology, Federal University of Technology, Akure, Nigeria. All the chemicals used were of analytical grade.

Sample preparation

Fresh green leafy vegetables were rinsed in water, dried on paper towel and the edible portions were separated from the inedible portion. The edible portions were chopped into almost equal small pieces or slices, mixed well and a portion (40 g) of the chopped vegetables was cooked by steaming in 200 ml of distilled water for 10 mins, while the other portion was not cooked. Fresh and cooked samples of the green leafy vegetables were then blended, centrifuged and filtered. The filtrates were stored in the refrigerator for subsequent analysis.

Determination of vitamin C content

Vitamin C content of the polar extracts was determined using the method of Benderitter et al. (1998). Briefly, 75 µl DNPH (2 g dinitro-

phenyl hydrazine, 230 mg thiourea and 270 mg CuSO4.5H $_2$ O in 100ml of 5mol Γ^1 H $_2$ SO $_4$) were added to 500 μ l reaction mixture (300 μ l of an appropriate dilution of the polar extract with 100 μ l 13.3% (TCA) and water). The reaction mixtures were subsequently incubated for 3 h at 37°C, then 0.5 ml of 65% H $_2$ SO $_4$ (v/v) was added to the medium, their absorbance was measured at 520 nm and the vitamin C content of the samples was subsequently calculated.

Determination of total phenol content

The total phenol content of the extracts was determined using the method reported by Singleton et al. (1999). Appropriate dilutions of the extracts were oxidized with 2.5mL of 10% Folin–Ciocalteau's reagent (v/v) and neutralized by 2.0 ml of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at 45°C and the absorbance was measured at 765 nm in the spectrophotometer. The total phenol content was subsequently calculated using gallic acid as standard.

Determination of total flavonoid content

The total flavonoid content of both extracts was determined using a slightly modified method reported by Meda et al. (2005). Briefly, 0.5 ml of appropriately diluted sample was mixed with 0.5 ml methanol, 50 μ l of 10% AlCl₃, 50 μ l of 1 mol l⁻¹ potassium acetate and 1.4 ml water and allowed to incubate at room temperature for 30 min. Thereafter, the absorbance of the reaction mixture was subsequently measured at 415 nm. The total flavonoid was calculated using quercetin as standard.

DPPH free radical scavenging ability

The free radical scavenging ability of the extracts against DPPH (1,1-diphenyl-2 picrylhydrazyl) free radical was evaluated as described by Gyamfi et al. (1999). Briefly, an appropriate dilution of the extracts (1 ml) was mixed with 1 ml of 0.4 mmol Γ^1 methanolic solution containing DPPH radicals. The mixture was left in the dark for 30 min and the absorbance was measured at 516 nm. The DPPH free radical scavenging ability was subsequently calculated with respect to the reference (which contains all the reagents without the test sample).

2,2-Azinobis (3-ethylbenzo-thiazoline-6-sulfonate (ABTS) radical scavenging ability

The ABTS* scavenging ability of the extracts was determined according to the method described by Re et al. (1999). ABTS* was generated by reacting an ABTS aqueous solution (7 mmol Γ^{-1}) with $K_2S_2O_8$ (2.45 mmol Γ^{-1} , final concentration) in the dark for 16 h and adjusting the Abs 734 nm to 0.700 with ethanol. 0.2 ml of appropriate dilution of the extract was added to 2.0 ml ABTS* solution and the absorbance were measured at 734 nm after 15 min. The trolox equivalent antioxidant capacity was subsequently calculated.

Determination of reducing property

The reducing property of the extracts was determined by assessing the ability of the extract to reduce $FeCl_3$ solution as described by Oyaizu. (1986). A 2.5 ml aliquot was mixed with 2.5 ml of 200 mmol Γ^1 sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min and

Sample	Raw	Cooked	% Loss
T. triangulare	547.3 ± 0.9	198.2 ± 0.1	63.8
B. alba	499.1 ± 0.5	292.9 ± 0.4	41.3
S. biafrae	647.9 ± 0.6	437.5 ± 0.2	32.5
O. gratissimum	842.0 ± 0.3	638.4 ± 1.0	24.2
T. occidentalis	625.9 ± 0.5	331.4 ± 0.8	47.1
A. hybridus	321.4 ± 1.0	227.7 ± 0.3	29.2

477.7 ± 0.1

 361.6 ± 0.2

 789.3 ± 0.8

 655.4 ± 0.2

Table 1. Effect of cooking on the vitamin C content (mg ascorbic acid equivalent/ 100 g) of some tropical green leafy vegetables.

Values represent means of triplicate readings.

then 2.5 ml of 10% trichloroacetic acid was added. This mixture was centrifuged at 650 rpm for 10 min. 5 ml of the super-natant was mixed with an equal volume of water and 1 ml of 0.1% ferric chloride. The absorbance was measured at 700 nm. The ferric reducing antioxidant property was subsequently calculated using ascorbic acid as standard.

I. batata

C. aconitifolius

Fe²⁺ chelation assay

The Fe²⁺ chelating ability of the extracts were determined using a modified method of Minotti and Aust (1987) with a slight modification by Puntel et al. (1995). Freshly prepared 500 $\mu mol\ l^{-1}$ FeSO₄ (150 μl) was added to a reaction mixture containing 168 μL of 0.1mol l^{-1} Tris-HCl (pH 7.4), 218 μl saline and the extracts (0 - 25 μl). The reaction mixture was incubated for 5 min, before the addition of 13 μl of 0.25% 1, 10-phenanthroline (w/v). The absorbance was subsequently measured at 510 nm in a spectrophotometer. The Fe²⁺ chelating ability was subsequently calculated with respect to the reference (which contains all the reagents without the test sample).

Data analysis

The results of the three replicates were pooled and expressed as mean \pm standard error (S.E.). A one-way analysis of variance (ANOVA) and the least significance difference (LSD) were carried out (Zar, 1984). Significance was accepted at p \leq 0.05.

RESULTS AND DISCUSSION

In this study, the effect of cooking the ascorbic acid, total phenolics and antioxidant activities of some green leafy vegetables were assessed. Vitamin C is found in fruits, particularly fruits and juices and green leafy vegetables. They protect the body against cancer of the oesophagus, oral cavity and stomach. It also helps to maintain the blood vessel flexibility and improves circulation in the arteries of the smokers (Block et al., 1992; Nagy, 1980). Vitamin C is a water-soluble antioxidant which is in a unique position to "scavenge" aqueous peroxyl radicals before these destructive substances damage the lipids (Rice-Evans and Miller, 1995). The result of the vitamin C content of some tropical green leafy vegetable and the

effect of cooking on them are shown in Table 1. The vitamin C content of raw green leafy vegetables ranges from 321.4 mg/100 g (A. hybridus) to 842.0 mg/100a (O. gratissimum) and that of cooked ranges from 198.2 mg/100 g (T. triangulare) to 638.4 mg/100 g (O. gratissi*mum*). Moreover, of all the leafy vegetables analyzed, O. gratissimum leaf had the highest vitamin C content, these results were similar to what Oboh et al. (2008) reported for polar extracts of some tropical vegetables. This indicates that tropical green leafy vegetables are very rich in vitamin C. However, cooking of the vegetables cause a significant decrease (P < 0.05) in the vitamin C content of all the vegetables. The highest level of decrease occurs in *T. triangulare* where cooking caused 63.8% loss in the vitamin C content, while O. gratissimum (24.2%) had the least loss in vitamin C content. This decrease in vitamin C content agrees with earlier findings of Oboh (2005) on some tropical vegetables that reported 47.5 - 82.4% loss in vitamin C content during blanching of vegetables. It is well established that vitamin C content are destroyed during cooking due to the fact that they are not stable at high temperature (Nagy and Smooth, 1977).

39 5

44.8

Phenolic compounds can protect the human body from free radicals, whose formation is associated with the normal natural metabolism of aerobic cells. The antiradical activity of flavonoids and phenols is principally based on the structural relationship between different parts of their chemical structure (Rice-Evans, 1996). Natural polyphenols are capable of removing free radicals, chelating metal catalysts, activating antioxidant enzymes, reducing α - tocopherol radicals and inhibiting oxidases (Amic et al., 2003). The total phenol content is presented in Table 2. The total phenol content of the uncooked leafy vegetables ranged from 146.9 mg/100 g (T. triangulare) to 693.8 mg/100 g (O. gratissimum), while total phenol content of the cooked vegetables ranged from 272.8 mg/100 g (*T. triangulare*) to 1037.5 mg/100 q(O. gratissimum). The highest level of increase in total phenol occurs in *T. triangulare* where cooking caused 85.7% gain, while C. aconitifolius (18.3%) had the least gain in total phenol content. This indicates that most of the phenolic compounds trapped in fibre of green leafy

Sample	Raw	Cooked	% Gain
T. triangulare	146.9 ± 0.4	272.8 ± 0.7	85.7
B. alba	350.0 ± 0.5	540.6 ± 0.3	54.5
S. biafrae	253.1 ± 0.1	443.8 ± 0.2	75.3
O. gratissimum	693.8 ± 0.9	1037.5 ± 0.2	49.6
T. occidentalis	290.6 ± 0.4	403.1 ± 0.4	38.7
A. hybridus	198.1 ± 0.1	300.0 ± 0.6	34.0
I. batata	367.5 ± 0.6	531.3 ± 0.8	44.6
C. aconitifolius	241.1 ± 0.4	285.1 ± 0.3	18.3

Table 2. Effect of cooking on the total phenol content (mg gallic acid equivalent /100 g) of some tropical green leafy vegetables.

Values represent means of triplicate readings.

vegetables are actually more available in the cooked compared to the raw. The percent gain in the total phenol content during cooking may be due to the break down of tough cell walls and release of phenolic compounds trapped in the fibre of green leafy vegetables for easier absorption in the small intestine (Oboh and Rocha, 2007). This result agrees with earlier report by Dewanto et al. (2002), where ferulic acid, a phenolic found in the cell wall of grains such as corn, wheat and oats, doubled after 10 min of cooking and increased by as much as 900% after 50 min of cooking (Dewanto et al., 2002).

Flavonoids have antioxidant activity and could therefore lower cellular oxidative stress, which has been implicated in the pathogenesis of various neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (Rice-Evans et al., 1996; Amic et al., 2003). The high flavonoid contents of vegetables may have contributed to its medicinal properties. The total flavonoid, reported as quercetin equivalent antioxidant capacity (QEAC), is presented in Table 3. The total flavonoids content of the raw vegetables ranged from 8.2 mg/100g (T. triangulare) to 53.0 mg/100 g (O. gratissimum), while the flavonoids content of cooked vegetables ranged from 12.9 mg/100 g (T. triangulare) to 57.4 mg/100 g (O. gratissimum). Therefore, total flavonoids of cooked vegetables were higher than total flavonoids of raw vegetables, indicating a possible release of some flavonoids during the cooking of the green leafy vegetables. However, the raw and cooked O. gratissimum leaves had the highest flavonoid contents of all the leafy vegetables analyzed. T. triangulare leaf recorded the highest percentage change in total flavonoid content (57.2%) after cooking, of all the leafy vegetables analyzed. This indicates that some flavonoids are released during cooking. Furthermore, there was an agreement between the percent gain in total phenol and total flavonoid contents with cooking in T. triangulare leaf, having the highest percent gain in total phenolic and flavonoid contents out of all the vegetables tested. This finding agrees with many earlier reports where correlations were established between total phenolic and total flavonoid contents (Melo et al., 2006; Oboh et al., 2007; Oboh and Rocha, 2008).

The antioxidant activities of plant phytochemicals occur by preventing the production of free radicals or by neutralizing/scavenging free radicals produced in the body or reducing/chelating the transition metal composition of foods (Melo, 2006; Oboh et al., 2007). Prevention of the chain initiation step by scavenging various reactive species such as free radicals is considered to be an important antioxidant mode of action (Dastmalchi et al., 2006), the DPPH (1,1-diphenyl-2 picrylhydrazyl) free radical scavenging ability of raw and cooked extracts of the leafy vegetables are presented in Figure 1. The results revealed that DPPH radical scavenging ability significantly increased (P < 0.05) with cooking in all the green leafy vegetables. However, raw (61.8%) and cooked (92.7%) extracts of O. gratissimum had the highest DPPH radical scavenging ability respectively. This increase in radical scavenging ability could be attributed to the increase in the total phenol and flavonoid content of the green leafy vegetables (despite the decrease in vitamin C content). This shows that phenolic could be the main antioxidant phytochemical in leafy vegetables and vitamin C. This assertion agrees with several results where correlation were establish between the total phenol content of some plant foods and their antioxidant capacity (Crozier et al., 1997; Zhang and Hamauzu, 2004; Ismail et al., 2004; Sahlin et al., 2004; Stewart et al., 2000; Turkmen et al., 2005).

DPPH is frequently used in the determination of free radical scavenging ability. However, it has the limitation of colour interference and sample solubility. Therefore, the free radical scavenging ability of the vegetable extracts was further studied using a moderately stable nitrogen-centered radical species, ABTS* (2,2- azinobis (3-ethylbenzo-thiazoline- 6-sulfonate). The ABTS radical based model of free radical scavenging ability has the advantage of being more versatile as both non-polar and polar samples can be assessed and spectral interference is minimized as the absorption maximum used is 760 nm, a wavelength not normally encountered with natural pro-

Sample	Raw	Cooked	% Gain
T. triangulare	8.2 ± 0.2	12.9 ± 0.1	57.2
B. alba	36.4 ± 0.4	50.6 ± 0.2	38.9
S. biafrae	43.6 ± 0.9	55.1 ± 0.3	26.3
O. gratissimum	53.0 ± 0.5	57.4 ± 0.3	8.3

 39.9 ± 0.3

 21.1 ± 0.7

 33.0 ± 0.2

 51.1 ± 0.3

32.7 25.0

30.7

21.5

 30.1 ± 0.3

 16.9 ± 0.8

 25.2 ± 0.3

 42.1 ± 0.1

Table 3. Effect of cooking on the total flavonoid content (mg quercetin equivalent /100 g) of some tropical green leafy vegetables.

Values represent means of triplicate readings.

T. occidentalis

A. hybridus

I. batata C. aconitifolius

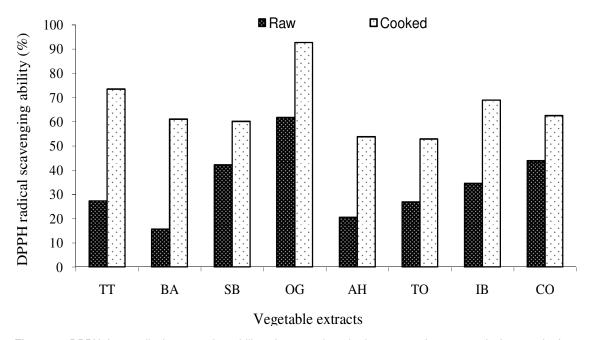


Figure 1. DPPH free radical scavenging ability of raw and cooked extracts of some tropical green leafy vegetables. Values represent means of triplicate readings. TT, *T. triangulare*; BA, *B. alba*; SB, *Senecio biafrae*; OG, *O. gratissimum*; TO, *T. occidentalis*; AH, *A. hybridus*; IB, *I. batata* and CA, *C. aconitifolius*

ducts (Re et al., 1999). ABTS* scavenging ability, reported as trolox equivalent antioxidant capacity (TEAC), is presented in Figure 2. The results also revealed that cooking caused a significant increase (P < 0.05) in the ABTS* scavenging ability of the leafy vegetables. Raw (87.3 mmol/100 g TEAC) and cooked (124.0 mmol/100 g TEAC) extracts of *O. gratissimum* leaf had the highest ABTS* scavenging ability.

This trend in the ABTS* scavenging ability of both the raw and cooked extract agrees with that of DPPH free radical scavenging ability. This confirms that *O. gratissimum* has the highest antioxidant activity of all the vegetables tested. However, it is worth noting that this increase in antioxidant activity with cooking, agrees with earlier report on the effect of cooking on the antioxidant properties of maize (Dewanto et al., 2002a), carrots

(Talcott et al., 2000) and tomatoes (Dewanto et al., 2002b). The increase in antioxidant activity will not be far fetch from the significant increase in the antioxidant phytochemicals (phenolics and flavonoid) that accompanied cooking.

Reducing power is a novel antioxidation defense mechanism. The 2 mechanisms that are available to affect this property are electron transfer and hydrogen atom transfer (Dastmalchi et al., 2007). The reducing powers of raw and cooked extracts were assessed based on their ability to reduce Fe (III) to Fe (II) and the results are presented in Figure 3 as ascorbic acid equivalent. The results revealed that cooked extracts had a higher reducing power than raw extracts in all the green leafy vegetables with *O. gratissimum* showing the highest reducing ability. The results of the reducing power agreed with the ABTS*

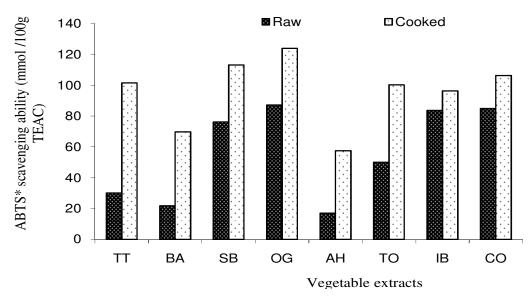


Figure 2. ABTS* radical scavenging ability of raw and cooked extracts of some tropical green leafy vegetables. Values represent means of triplicate readings. TT, *T. triangulare*; BA, *B. alba*; SB, *S. biafrae*; OG, *O. gratissimum*; TO, *T. occidentalis*; AH, *A. hybridus*; IB, *I. batata* and CA, *C. aconitifolius*

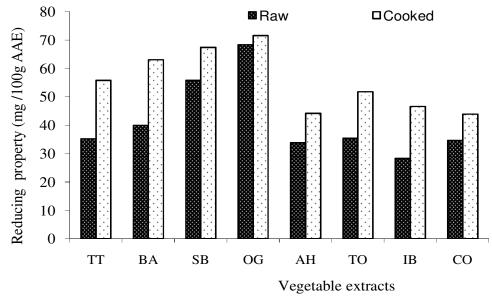


Figure 3. Ferric reducing antioxidant power (FRAP) of raw and cooked extracts of some tropical green leafy vegetables. Values represent means of triplicate readings. TT, *Talinium triangulare*; BA, *B. alba*; SB, *Senecio biafrae*; OG, *O. gratissimum*; TO, *T. occidentalis*; AH, *Amaranthus hybridus*; IB, *I. batata* and CA, *C. aconitifolius*

and DPPH free radical scavenging abilities earlier discussed, this goes on to confirm that cooking could increase antioxidant activity, as a result of the increase in total phenol and flavonoid content during cooking as shown in Tables 2 and 3. Furthermore, the fact that the decrease in the ascorbic acid could not cause a decrease in the antioxidant indices during cooking revealed that phenolic constituent may be the domineering antioxidant in the

vegetables. However, of all the tropical leafy vegetables analyzed, *O. gratissimum* leaf appears to have the highest antioxidant properties, as typified by the highest radical scavenging ability (DPPH and ABTS*) and reducing power.

The ability of antioxidants to chelate and deactivate transition metals prevents such metals from participating in the initiation of lipid peroxidation and oxidative stress

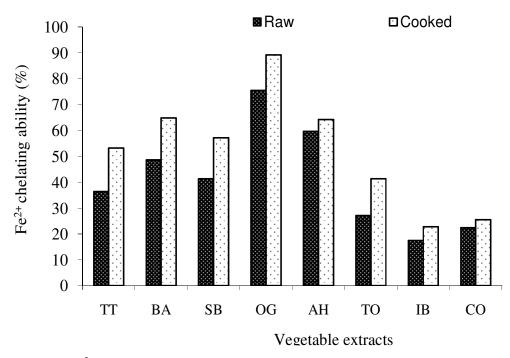


Figure 4. Fe²⁺ chelating ability of raw and cooked extracts of some tropical green leafy vegetables. Values represent means of triplicate readings. TT, *T. triangulare*; BA, *B. alba*; SB, *S. biafrae*; OG, *O. gratissimum*; TO, *T. occidentalis*; AH,

through metal-catalyzed reaction (Dastmalchi et al., 2007). The ability of the extracts to chelate transition metals is therefore considered to be due to an antioxidant mechanism (Dastmalchi et al., 2007; Oboh et al., 2007).

Fe (II) chelating ability of the extracts was determined and the results are presented in Figure 4. The results reveal that all the extracts chelate Fe (II). However, the cooked extracts had a significantly higher (P < 0.05) Fe (II) chelating ability than the raw extracts. The trend in the Fe (II) chelating ability is similar to that of the DPPH radical scavenging, ABTS* radical scavenging ability and reducing power. The total antioxidant capacity is a combination of different antioxidant mechanisms, including free radical scavenging ability, reducing power and Fe (II) chelating ability. Since there was increase in the various antioxidant mechanisms during cooking of the green leafy vegetables, it could be inferred that cooking could increase the antioxidant capacity of tropical green leafy vegetables. This increase in antioxidant capacity during the cooking of the leafy vegetables could be attributed to the significant increase in the total phenol and flavonoids during cooking.

Conclusion

Cooking is an indispensable prerequisite in order to obtain safe and high-quality food products. Cooked vegetables have much better hygienic quality and due to chemical reactions during cooking, they are much better digestible and have an increased nutritional value. How-

ever, cooking may affect antioxidant status of tropical green leafy vegetables due to the release of more phenolic compounds and destruction or creation of redoxactive metabolites.

REFERENCES

Alia M, Horcajo C, Bravo, Goya L (2003). Effect of grape antioxidant dietary fiber on the total antioxidant capacity and the activity of liver antioxidant enzymes in rats. Nutr. Res. 23: 1251-1267.

Amic D, Davidovic-Amic D, Beslo D, Trinajstic N (2003). Structureradical scavenging activity relationship of flavonoids. Croatia Chem. Acta 76: 55-61.

Benderitter M, Maupoil V, Vergely C, Dalloz F, Briot F, Rochette L (1998). Studies by electron paramagnetic resonance of the importance of iron in the hydroxyl scavenging properties of ascorbic acid in plasma: effects of iron chelators. Fundam. Clin. Pharmacol. 12: 510–516

Block G, Patterson B, Subar A (1992). Fruits vegetables and cancer prevention. A review of epidemiological evidence. Nutr. Cancer 18: 1-29.

Chu Y, Sun J, Wu X, Liu RH (2002). Antioxidant and antiproliferative activity of common vegetables. J. Agric. Food Chem. 50: 6910-6916.

Crozier A, Lean M E J, McDonald MS,Black C (1997). Quantitative analysis of the flavonoid content of commercial tomatoes, onions, lettuce, and celery. J. Agric. Food Chem. 45: 590-595.

Dastmalchi K, Dorman HJD, Kosar M, Hiltunen R (2007). Chemical composition and *in vitro* antioxidant evaluation of a water soluble Moldavian balm (*Dracocephalum moldavica* L.) extract. Lebensm Wiss Technol 40: 239–248.

Dewanto V, Wu X, Liu RH (2002a). Processed sweet corn has higher antioxidant activity. J. Agric. Food Chem. 50 (17): 4959-4964.

Dewanto V, Wu X, Adom KK, Liu RH (2002b). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J. Agric. Food Chem. 50(10): 3010-3014.

Gülçin I, Oktay M, Kirecci E, Kufrevioglu O I (2003). Screening of anti-

- oxidant and antimicrobial activities of anise (*Pimpinella anisum* L.) seed extracts. Food Chem. 83: 371-382
- Gyamfi MA, Yonamine M, Aniya Y (1999) Free-radical scavenging action of medicinal herbs from Ghana: *Thonningia sanguinea* on experimentally-induced liver injuries. Gen. Pharmacol. 32: 661-667.
- Ismail A, Marjan ZM, Foong CW (2004). Total antioxidant activity and phenolic content in selected vegetables. Food Chem. 87: 581-586.
- Meda A, Lamien CE, Romito M, Millogo J, Nacoulma OG (2005). Determination of the total phenolic, flavonoid and praline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chem. 91:571–577
- Melo EA, Lima VLAG, Maciel MIS, Caetano ACS, Leal FLL (2006). Polyphenol, ascorbic acid and total carotenoid contents in common fruits and vegetables. Braz. J. Food Technol. 9: 89-94.
- Minotti G, Aust SD (1987). An investigation into the mechanism of citrate-Fe²⁺- dependent lipid peroxidation. Free Rad. Biol. Med. 3: 379-387
- Nagy S, Smooth JM (1977). Temperature and storage effects on percent retention and percent U.S. recommended dietary allowances of vitamin C in canned single-strength orange juice. J. Agric. Food Chem. 25(1): 135-138.
- Nagy S (1980). Vitamin C contents of Citrus fruit and their products: A review. J. Agric. Food Chem. 28(1): 8-18.
- Oboh G, Akindahunsi AA (2004). Change in the ascorbic acid, total phenol and antioxidant activity of some sun-dried green leafy vegetables in Nigeria. Nutr. Health 18: 29-36.
- Oboh G (2005). Effect of blanching on the antioxidant property of some tropical green leafy vegetables. Lebensm Wiss Technol 38: 513-517.
- Oboh G, Rocha JBT (2007a). Antioxidant in Foods: A New Challenge for Food processors. Leading Edge Antioxidants Research, Nova Science Publishers Inc. New York US, pp. 35-64.
- Oboh G, Rocha JBT (2007b). Polyphenols in Red pepper [Capsicum annuum var. aviculare (Tepin)] and their protective effect on some pro-oxidants induced lipid peroxidation in Brain and Liver. Eur. Food Res. Technol. 225: 239-247.
- Oboh G, Puntel RL, Rocha JBT (2007). Hot pepper (*Capsicum annuum*, *Tepin* and *Capsicum chinese*, *Habanero*) prevents Fe²⁺ induced lipid peroxidation in Brain In vitro. Food Chem. 102: 178-185.
- Oboh G, Rocha JBT (2008). Water extractable phytochemicals from *Capsicum pubescens* (tree pepper) inhibit lipid peroxidation induced by different pro-oxidant agents in brain: in vitro. Eur. Food Res. Technol. 226: 707-713.
- Oboh G, Raddatz H, Henle T (2008). Antioxidant properties of polar and non-polar extracts of some tropical green leafy vegetables. J Sci. Food Agric. 88: 2486-2492.
- Oyaizu M (1986). Studies on products of browning reaction: antioxidative activity of products of browning reaction prepared from glucosamine. Jpn. J. Nutr. 44: 307–315.
- Puntel RL, Nogueira CWand Rocha JBT (2005). Krebs cycle intermediates modulate thiobarbituric acid reactive species (TBARS) production in rat brain *in vitro*. Neurochem. Res. 30: 225-235.

- Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999). Antioxidant activity applying an improved ABTS radical cation decolorisation assay. Free Rad. Biol. Med. 26: 1231-1237.
- Rehman ZU, Islam M, Shah WH (2003). Effect of microwave and conventional cooking on insoluble dietary fibre components of vegetables. Food Chem. 80: 237-240
- Rice-Evans C, Miller NJ (1995). Antioxidants: the case for fruit and vegetables in the diet. Br. Food J. 97: 35-40.
- Rice-Evans C, Miller NJ, Paganga G (1987). Structure–antioxidant activity relationships of flavonoids and phenolic acids. Free Rad. Biol. Med. 20: 933-956.
- Sahlin E, Savage GP, Lister CE (2004). Investigation of the antioxidant properties of tomatoes after processing. J. Food Comp. Anal. 17: 635-647.
- Singleton VL, Orthofer R, Lamuela-Raventos RM (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteau's reagent. Methods Enzymol. 299: 152-178.
- Stewart AJ, Bozonnet S, Mullen W, Jenkins GI, Michael EJ, Crozier A (2000). Occurrence of flavonols in tomatoes and tomato-based products. J. Agric. Food Chem. 48: 2663-2669.
- Sun J, Chu Y, Wu X, Liu R (2002). Antioxidant and antiproliferative activities of common fruits. J. Agric. Food Chem. 50: 7449-7454.
- Turkmen N, Sari F, Velioglu Y (2005). The effect of cooking methods on total phenols and antioxidant activity of selected green vegetables. Food Chem. 93: 713-718.
- Talcott ST, Howard LR, Brenes CH (2000). Contribution of peridermmaterial and blanching time to the quality of pasteurized peach puree. J. Agric. Food Chem. 48(10): 4590-4596
- Zar JH (1984) Biostatistical Analysis, Prentice-Hall, Inc., USA. p. 620.
- Zhang Z, Chang Q, Zhu M, Huang Y, Ho WKK, Chen Z (2001). Characterization of antioxidants present in hawthorn fruits. J. Nutr Biochem. 12: 144-152
- Zhang D, Hamauzu Y (2004). Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking. Food Chem. 88: 503-509.