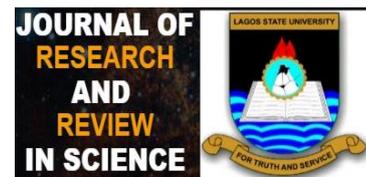


ORIGINAL RESEARCH

ANTIOXIDANT CAPACITY OF SELECTED LOCALLY AVAILABLE VEGETABLES

Oluwatosin Adu¹, Olabisi Ogunrinola¹, Gbemisola Saibu¹, Moses Akiibinu², Godswill Njiaka², Omolade Fajana¹, Sedoten Ogun¹, Babajide Elemo¹

¹Department of Biochemistry, Lagos State University, Ojo, Lagos, PMB 0001, LASU Post Office, Ojo, Lagos.

²Department of Chemistry and Biochemistry, Caleb University, Imota, Lagos.

Correspondence

Oluwatosin Benedict Adu, Department of Biochemistry, Faculty of Science, Lagos State University, Ojo, Lagos-Nigeria.
Email: oluwatosin.adu@lasu.edu.ng

Abstract:

Introduction: Fairly large scale analyses have been done to evaluate the antioxidant capacity of vegetables and correlation between the various methods of analysis. However, very little has been done to relate the antioxidant capacities of these vegetables with their pH values. This study was carried out to determine the antioxidant capacity of selected widely consumed and locally available vegetables in Nigeria and relate the antioxidant capacity of these vegetables to their pHs.

Methods: The vegetables were grouped into “green vegetables”-cucumber cabbage, lettuce and spring onions and “red vegetables”-tomato, scotch bonnet, cayenne pepper and carrot. The pHs of these vegetables were determined. Two different methods-2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay and Ferric reducing antioxidant power (FRAP) assay were used to determine the antioxidant capacity.

Results: Results of the pH measurements of “green vegetables” showed a range of 5.84-6.72 while the pH of “red vegetables” ranged from 4.87-5.66. All vegetables used in this study had pH less than 7 with relatively high antioxidant capacity. Scotch bonnet had consistently high antioxidant activity regardless of the method employed. There was a significant negative relationship ($P<.05$; $r = -0.365$) between the pH and DPPH radical scavenging activity and between the pH and total antioxidant capacity ($P<.05$ $r = -0.351$). There was no significant correlation ($P<.05$) between DPPH radical scavenging and total antioxidant capacity of all vegetables.

Conclusion: The pHs of these vegetables were within the acidic range, and antioxidant potential of these vegetables increased with increasing acidity.

Key words: Antioxidants, vegetables, pH, DPPH, FRAP

All co-authors agreed to have their names listed as authors.

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1. INTRODUCTION

Nutraceuticals such as vitamins, minerals and phenolic compounds occur in fruits and vegetables [1]. They are required in small amounts and are essential parts of the diet since the body cannot adequately synthesize them to meet its demand. World Health Organization (WHO) reported that the occurrence of some diseases such as cardiovascular diseases and some forms of cancer have been linked to low consumption of fruit and vegetables which has also led to lots of deaths worldwide [2].

Fruit and vegetable intake is necessary for growth, proper function [2] and protection of the body against free radicals [3, 4]; being excellent sources of natural antioxidants. These antioxidants help to inactivate reactive oxygen species and prevent oxidative damage. Free radicals have been established to be a major contributor to non communicable diseases such as cancer and cardiovascular diseases [5, 6]. Some antioxidants are naturally occurring in human cells such as catalase, superoxide dismutase and glutathione peroxidase. Dietary antioxidants which includes vitamin C, flavonoids vitamin E, carotenoids and polyphenols, have received much attention over the years with respect to disease prevention [4, 7, 8].

pH is a logarithmic scale used to determine the acidity or basicity of an aqueous solution. Solutions with a pH less than 7 are acidic and solutions with a pH greater than 7 are basic. pH in the body varies greatly from one part to another depending on the function of the organ with the highest acidity in the stomach (pH of 1.35 to 3.5) to aid in digestion and protect against opportunistic microbial organisms [9].

In recent times, there have been controversies on the necessity, or otherwise of consuming alkaline foods; with some authors suggesting that continuous and consistent consumption of acidic foods may lead to long term problems, while consumption of alkaline rich foods will support health and help the body to remove the stress of an acidic lifestyle [10, 11]. Fruits and vegetables have been proposed to be associated with a greater degree of alkalinity [12, 13]. The concept that alkaline foods prevent and cure diseases are marketed to the general public [14]. Marketers claim that alkaline foods and related commercial products counteract acidity (which has been found to support the growth of cancer cells and tumors [15, 16]), help the body regulate its pH, and thus prevent diseases [17-19]. While alkaline foods are promoted to correct the acidic state of the body, the American Institute of Cancer Research and the Canadian Cancer Society have stated that the body tightly regulates systemic pH and food choices will only affect urine pH and not body acidity [20]. Studies show that while urine pH changes in response to diet changes, blood pH does not [20].

Nigeria is found in the tropics, where the climate is seasonally damp and very humid with abundance of fruits and vegetables. These foods are not only produced and sold in the villages but are brought to towns for economic reasons. Some of these vegetables are currently cultivated in small quantities in towns by immigrants from villages making them

readily available all through the year, these include: cucumber (*Cucumis sativus*), cabbage (*Brassica oleracea*), lettuce (*Lactuca sativa*), spring onions (*Allium fistulosum*), tomato (*Solanumly copersicum*), scotch bonnet ("rodo") (*Capsicum chinense*), cayenne pepper ("sombo") (*Capsicum frutescens*) and carrot (*Daucus carota*).

Although lots of work have been done on evaluating the antioxidant capacity of vegetables, and correlation between the various methods of analysis [2, 7, 21-25], very little has been done to relate the antioxidant capacities of these vegetables with their pH. The aim of this study was to determine the antioxidant capacity of selected widely consumed and locally available vegetables in Nigeria and relate the antioxidant capacity of these vegetables to their pH.

2. MATERIAL AND METHODS

2.1 PLANT MATERIALS

Fresh vegetable samples were gotten in the month of July from Sabo market, Ikorodu Local Government of Lagos state, Nigeria. These samples include "green" vegetables - cucumber (*Cucumis sativus*), cabbage (*Brassica oleracea*), lettuce (*Lactuca sativa*) and spring onions (*Allium fistulosum*) and "red" vegetables - tomato (*Solanum lycopersicum*), scotch bonnet (*Capsicum chinense*), cayenne pepper (*Capsicum frutescens*) and carrot (*Daucus carota*).

2.2 CHEMICALS

2,4-Dinitrophenylhydrazin (DPPH), CH₃OH (Methanol), CH₃COONa.3H₂O (Sodium Acetate (Trihydrate)), C₆H₈O₆ (Ascorbic acid) were purchased from Sigma-Aldrich. All other chemicals used, including the solvents, were of analytical grade.

2.3 EXTRACT PREPARATION

The vegetable samples were rinsed with water, sliced, blended and sieved with a white muslin cloth. The extracts were further filtered using filter paper to get the aqueous extracts. The extracts were preserved in a freezer at -20°C until they were ready for analysis.

2.4 DETERMINATION OF pH

A standard procedure was used in determining the pH of the extracts using a PHS-25pH metre

2.5 ANTIOXIDANT ANALYSIS

Antioxidant parameters measured were DPPH radical scavenging activity and total antioxidant activity (Ferric reducing antioxidant power (FRAP) Assay).

2.5.1 DPPH RADICAL SCAVENGING ASSAY

DPPH radical scavenging assay was evaluated with the method of Liyana-Pathirana and Shahidi [26]. 0.012g of DPPH was dissolved in 300ml of methanol

and 1.0ml was added to 1.0ml of extract in methanol containing 0.02-0.1mg of the extract. The reaction was vortexed then left at room temperature for 30mins. The absorbance was taken at 530nm using UV-Visible Spectrophotometer. Ascorbic acid was used as reference.

DPPH radical scavenging activity (%) =

$$\frac{\text{Abs of control} - \text{Abs of Sample}}{\text{Abs of control}} \times 100\%$$

2.5.2 FERRIC REDUCING ANTIOXIDANT POWER (FRAP)

FRAP was determined by a modified method of Benzie and Strain [19]. Plant extracts (1 μM) were allowed to react with 200 μl of the FRAP solution for 30 min in the dark. Absorbance was measured at 593 nm. Results were expressed in μM Fe (II)/g dry mass and compared with ascorbic acid [27].

2.6 STATISTICAL ANALYSIS

Data were analysed using Statistical package for social science version 20. Values of all parameters were expressed as mean \pm standard error of four samples determination. Results were subjected to one-way analysis of variance (ANOVA) at 95% confidence limit and for *post hoc* analysis, Tukey's honest significant difference test was used.

3. RESULTS

Results of the pH measurements of vegetables (Fig 1) ranged from 4.87-6.74. Cucumber had the highest pH with a value of 6.74 which was followed by lettuce (6.3), spring onions (5.87), cabbage (5.84), cayenne pepper (*sombo*) (5.66), carrot (5.51), scotch bonnet (*rodo*) (5.46) and tomato (4.87). The pH of cucumber was significantly higher ($P < 0.05$) than all the other vegetables and the pH of tomato was significantly lower ($P < 0.05$) than that of all other vegetables. Generally, the pH values of green vegetables were significantly higher than those of red vegetables.

The DPPH radical scavenging activity of vegetables (Fig 2) was in the range of 45.80% - 81.03%. There was no clear cut distinction between DPPH scavenging activity of green and red vegetables. Scotch bonnet had the highest DPPH scavenging activity with a value of 81.03% which was followed by tomatoes (79.65), spring onion (78.77), cabbage (78.77), lettuce (66.78), carrot (57.83), cucumber (53.42) and cayenne pepper (45.80). The DPPH scavenging activity of scotch bonnet, tomatoes and spring onion were significantly higher ($P < 0.05$) than all

other vegetables. All vegetables except carrot and cucumber were significantly higher than cayenne pepper. None of the vegetables-green or red- had higher radical scavenging activity compared to ascorbic acid (98%).

The total antioxidant activity of vegetables (Fig 3) ranged from 575 $\mu\text{mol/L}$ - 1365 $\mu\text{mol/L}$. The extractives of scotch bonnet demonstrated the highest total antioxidant power with a value of 1365 $\mu\text{mol/L}$ which was followed by cayenne pepper (1290 $\mu\text{mol/L}$), carrot (900 $\mu\text{mol/L}$), spring onion (765 $\mu\text{mol/L}$), cabbage (745 $\mu\text{mol/L}$), tomatoes (675 $\mu\text{mol/L}$), cucumber (625 $\mu\text{mol/L}$) and lettuce (575 $\mu\text{mol/L}$). Scotch bonnet and cayenne pepper were significantly higher ($P < 0.05$) than all other vegetables. There was no significant difference between lettuce, cucumber, tomatoes, cabbage and spring onion.

The correlation between pH values of the selected "green vegetables" and their antioxidant potentials is shown in Table 1. There was a significant negative relationship ($P < 0.05$ $r = -0.788$) between the pH and DPPH radical scavenging activity and between the pH and total antioxidant capacity ($P < 0.05$; $r = -0.611$).

There was no significant correlation ($P < 0.05$) between DPPH radical scavenging and total antioxidant capacity of "green vegetables".

There was a significant negative relationship ($P < 0.05$ $r = -0.609$); between the pH and DPPH radical scavenging activity of "red vegetables" (Table 1) but a significant positive relationship ($P < 0.05$ $r = 0.717$) between the pH and total antioxidant capacity. There was however no significant correlation ($P < 0.05$) between DPPH radical scavenging and Total Antioxidant of "red vegetables".

Overall, There was a significant negative relationship ($P < 0.05$ $r = -0.365$) between the pH and DPPH radical scavenging activity and between the pH and total antioxidant capacity ($P < 0.05$ $r = -0.351$). There was no significant correlation ($P < 0.05$) between DPPH radical scavenging and total antioxidant capacity of all vegetables.

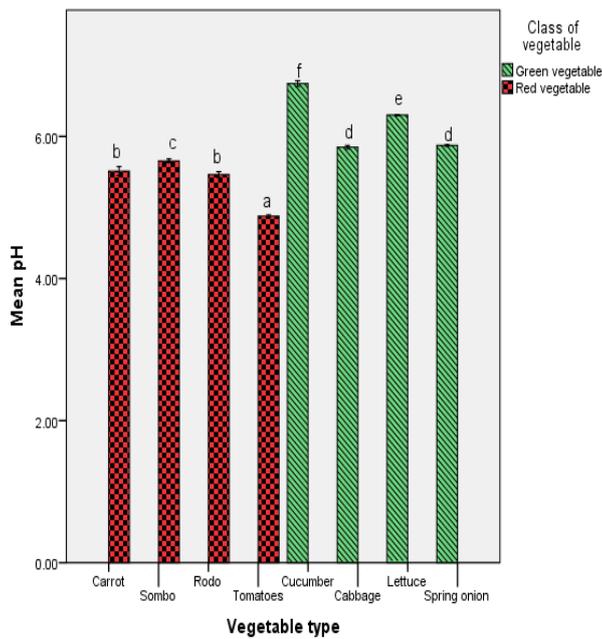


Fig. 1. pH values of all vegetables

ab Bars with different superscripts are significantly different at $p < 0.05$

Values are represented as mean \pm standard error of four samples determination

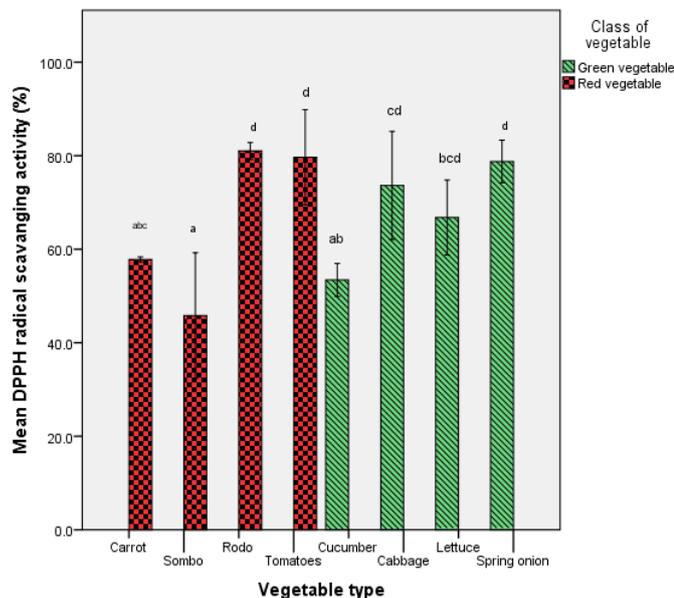


Fig. 2. DPPH radical scavenging activity of all vegetables

ab Bars with different superscripts are significantly different at $p < 0.05$

Values are represented as mean \pm standard error of four samples determination

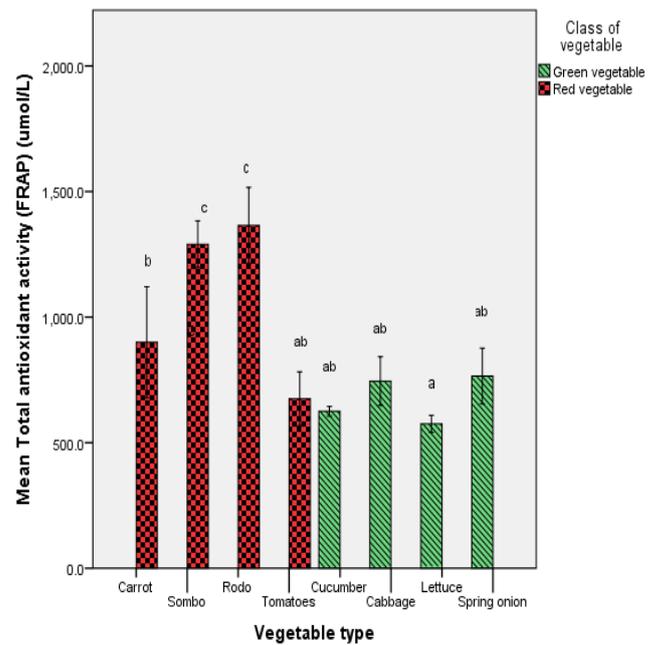


Fig. 3. Total antioxidant activity (FRAP) of all vegetables

ab Bars with different superscripts are significantly different at $p < 0.05$

Values are represented as mean \pm standard error of four samples determination

Table 1. Correlations between pH and antioxidant activities of vegetables

Correlation	R	p
“Green” vegetables		
pH vs DPPH	-0.788**	0.000
pH vs FRAP	-0.611*	0.012
DPPH vs FRAP	0.418	0.107
“Red” vegetables		
pH vs DPPH	-0.609*	0.012
pH vs FRAP	0.717**	0.002
DPPH vs FRAP	-0.233	0.386
“All” vegetables		
pH vs DPPH	-0.365*	0.040
pH vs FRAP	-0.351*	0.049
DPPH vs FRAP	-0.127	0.487

p=Sig. (2-tailed)r=Pearson Correlation

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

4. DISCUSSION

It is well established that no single testing method can adequately provide complete information on the antioxidant profile of a sample examined because they all present limitations. Therefore different methods are used for analyzing antioxidant activity, with varying concepts and mechanisms of action [24, 25]. The DPPH method gives a broad idea about the radical

scavenging ability but does not differentiate between the radical species [24] while FRAP actually measures only the reducing capability based upon ferric ion [28]. Both methods are very simple and convenient for the screening of antioxidant activity [24, 28]. In this study, the DPPH radical scavenging activity and ferric reducing antioxidant power was used to measure antioxidant capacity.

The DPPH radical scavenging assay is based on the use of the stable free radical diphenylpicrylhydrazyl [29]. It is a well-known method that is often used to test the free radical scavenging power of foods and their ability to act as hydrogen donors [30, 31]. It has been reported that DPPH free radicals are not affected by certain side reactions such as metal ion chelation and enzyme inhibition [32]. All the vegetables used in this study, with the exception of cucumber and cayenne pepper, had DPPH radical scavenging activity above 60%. There was no clear-cut distinction in DPPH scavenging activity between green and red vegetables. Scotch bonnet had a very high DPPH scavenging activity which was also reported by Kevers et al [23], whereas cucumber and carrot had the lowest antioxidant activity, similar to the report of Kevers et al [23]; Souri et al [33]; Bayili et al [21]; and Tiveron et al [25]. Ascorbic acid and phenolic contents of fruits and vegetables have been linked to their DPPH radical scavenging activity [7, 25, 33]. Tiveron *et al* [17]; Kevers et al [23] and van den Brandt [34] reported that some of these vegetables are rich in ascorbic acid and this may have contributed to the antioxidant activity of these vegetables.

The reducing ability of a compound may indicate its possible antioxidant potential [35]. The ranking order of total antioxidant activity (FRAP) of green and red vegetables in this study is similar to the ones reported in earlier studies [21]. There was a clear distinction between green and red vegetables with red vegetables having the greatest ability to reduce Fe^{3+} to Fe^{2+} but this contradicts the result of Tiveron et al [17] carried out on vegetables from Brazil, and in which lettuce had higher reducing power than carrot. This may be due to the differences in the solvent extracts. From existing literature, several factors such as temperature, seasonality, water availability, UV radiation, soil nutrients, pollution, and pathogen attack can affect the content of secondary metabolites in fruits and vegetables [25, 36]. FRAP assay was shown to have high correlation with ascorbic acid and total phenolics [37].

Fruit and vegetable extracts vary not only in the composition and quality of antioxidants, but also of natural acids. This suggests that fruit and vegetable extracts have varying pH [38]. In this study, the pH of red vegetables (4.87-5.66) was significantly more acidic than those of green vegetables (5.84-6.74). This could be as a result of higher content of ascorbic acid and phenolic acids as observed in previous work [21, 39, 40]. Very little information exists on the relationship between pH and antioxidant activity of plant extracts. In this study, there was a significant negative correlation between pH and both DPPH scavenging

activity ($p < 0.05$; $r = -0.365$) and ferric reducing power ($p < 0.05$; $r = -0.351$) of the vegetables; indicating that with increase in pH, there was a reduction in antioxidant activity. This is contrary to what was reported by Pekal and Pyszynska [38] for tea infusions. Their justification was based on the postulation of Dawidowicz and Olszowy [41] that the change of hydrogen ion concentration caused the change of the mechanism of scavenging process of DPPH radicals by phenolic compounds. However in the findings of Bayliak et al [42], plant aqueous extracts were able to scavenge H_2O_2 effectively at acidic, than at alkaline pH which is similar to our findings. Decreased H_2O_2 and DPPH scavenging at alkaline pH was related to the unstableness of many phenolic compounds at that pH [43] leading to a decline in antioxidant activity. The possible reason for our own observation is currently under investigation.

There was a positive correlation between DPPH scavenging activity and the ferric reducing antioxidant power, but this relationship was not significant. Tiveron et al [25] reported a strong correlation between DPPH and FRAP. This could be because of interference caused by color in some of the extracts, and slow development of color in FRAP assay. Slow development of color has been reported in other studies [44, 45]. This indicates the involvement of several antioxidants in the medium acting under different kinetic conditions [46].

5. CONCLUSION

All vegetables used in this study had pH less than 7 and relatively high antioxidant capacity. Overall, scotch bonnet had consistently high antioxidant activity regardless of the method employed. Antioxidant potential of these vegetables increased with increasing acidity.

AUTHORS' CONTRIBUTIONS

Author OA, designed the study and performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author OO, GS, MA, OF and BE were involved in the study design and experimental protocol. Author GN and SO carried out the experiment and managed the literature searches. All authors read and approved the final manuscript.

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