Journal of Chemistry and Nutritional Biochemistry
https://jcnb.sabapub.com
E ISSN: 2709-5932

2025 Volume 6, Issue 2: 1–17
DOI: https://doi.org/10.48185/jcnb.v6i1.1421

Relationship between pH and antioxidant capacity of selected locally available vegetables

Sedoten Ogun[1,2], Mosunmola Akanni[1,3], Gbemisola Saibu[1], Oluwatosin Adu[1*]

[1]Department of Biochemistry, Lagos State University, Ojo, Lagos, PMB 0001, LASU Post Office, Ojo, Lagos, Nigeria
[2]Department of Food Science and Technology, University of Nebraska-Lincoln, Lincoln, Nebraska, USA
[3] Department of Biochemistry, Covenant University, Ota 112104, Ogun State, Nigeria

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

Received: 07.12.2024 • Accepted: 24.03.2025 • Published: 12.04.2025 • Final Version: 12.04.2026

Abstract: In recent times, there have been controversies on the consumption of alkaline foods; with some authors suggesting that consistent consumption of acidic foods may lead to long-term health challenges, while consumption of alkaline foods will support health and help the body to remove the stress of an acidic lifestyle. Fruits and vegetables have been proposed to be associated with a greater degree of alkalinity. Thus, there is a need to determine the antioxidant capacities of selected vegetables with a particular focus on understanding how their pH influences antioxidant activity. This study evaluated 15 locally available vegetables: Fluted pumpkin leaves (Ugwu), Jute mallow (Ewedu), Bitter leaf, African basil (Scent leaf), Amaranthus, Cabbage, Lettuce, Okro, Cucumber, Cayenne pepper (Sombo), Habanero pepper (Rodo), Bell pepper (Tatase), Tomato, Carrot and Spring onions. The pH of the fresh and freeze-dried samples, Phytochemical analysis (Total phenolics and Ascorbic acid content) and Antioxidant Analysis−1, 1-diphenyl-2- picryl-hydrazil (DPPH) radical scavenging activity, Ferric reducing antioxidant power (FRAP) assay, Nitric Oxide (NO) scavenging activity and Total antioxidant activity (TAC)−were determined. Correlation analysis was done to establish relationships between pH, phytochemical constituents and antioxidant capacities of the selected vegetables. Results revealed that most of the fresh and freeze-dried vegetables had pH less than or greater than 7 respectively. The vegetables with higher concentrations of phytochemicals−ascorbic acid and total phenolic− had higher antioxidant capacities. While there was no significant correlation between pH and DPPH IC_{50} there was a significant negative correlation between pH of freeze-dried samples and NO IC_{50}. Additionally, the pH of both fresh and freeze-dried vegetables was significantly negatively correlated with the TAC. This indicated that as the pH of the vegetables increased their antioxidant capacity reduced. Thus, the antioxidant potentials of these vegetables increased with increasing acidity which is contrary to popular belief.

Keywords: Antioxidant, pH, Phytochemical, Vegetable, DPPH, FRAP, Nitric oxide, Total phenolics, Ascorbic acid

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

1. Introduction

The impact of dietary choices particularly the consumption of alkaline or acidic foods has sparked a series of controversies with some authors suggesting that continuous and consistent consumption of acidic foods may lead to long-term health challenges, while consumption of alkaline-rich foods will support health and help the body to remove the stress of an acidic lifestyle (Mohammed et al., 2023; Passey, 2017; Remer & Manz, 1995; Suthar & Verma, 2014). This discussion is particularly significant in the context of oxidative stress associated with the overproduction of free radicals/oxidants in the body. Oxidative stress has been linked to the development of various diseases such as cancer and neurological disorders (Poprac et al., 2017). Although the body has an endogenous antioxidant defense system comprising of enzymes such as catalase, gluthathione peroxidase, superoxide dismutase, oxidative stress arises when there is an imbalance between the generation and elimination of these free radicals leading to cellular damage and diseases (Zhu et al., 2023). Antioxidants are known for their role in inhibiting the oxidation of other molecules preventing oxidative damage to body cells and prevent the onset of various diseases (Gulcin, 2020).

There is a growing concern about the safety of synthetic antioxidants and an increasing preference for natural antioxidants (Akbarirad et al., 2016). Currently, high doses of synthetic antioxidants like butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT) and tertiary butylated hydroquinone (TBHQ) have been linked to being potential carcinogens (Akbarirad et al., 2016; Felter et al., 2021). Hence, strong restrictions have been placed on their application. There is also an increasing consumer preference for naturally occurring antioxidants (Akbarirad et al., 2016; Mitterer-Daltoe et al., 2020) like vitamin C, vitamin A, vitamin E, lycopene, vitamin B2 (riboflavin), beta-carotene commonly found in varieties of fruits and vegetables (Akbarirad et al., 2016). Cancer, aging, diabetes, liver disease, heart disease, arthritis, AIDS, Alzheimer's disease and multiple sclerosis are just a few of many conditions that are thought to be associated with an inadequate intake of antioxidants. Studies have shown the existence of a significant correlation between the intake of fruits and vegetables and the decrease in mortality and morbidity due to degenerative processes caused by oxidative stress (Birt et al., 2001; Dragsted et al., 2004).

While numerous studies have examined the antioxidant capacities of fruits and vegetables globally (Amarowicz et al., 2004; Bayili et al., 2011; Dumbravă et al., 2011; Venkatachalam et al., 2014), most studies in Nigeria have focused on leafy vegetables. There remains a significant gap in understanding the antioxidant properties of other vegetable types, particularly in the context of their pH. This is noteworthy, as total content and relative proportions of secondary metabolites, which influence antioxidant activity, vary across time, location, and environmental conditions (Gobbo-Neto & Lopes, 2007). Fruits and vegetables have been proposed to be associated with a greater degree of alkalinity (Remer & Manz, 1995; Tucker et al., 2001) but very little information exists on the relationship between pH and the antioxidant activity of fruits and vegetables. Thus, the aim of this study was to determine the *in vitro* antioxidant capacities of selected locally available vegetables with a particular focus on understanding how their pH influences antioxidant activity. By investigating the relationship between the antioxidant potential of these vegetables and their pH, this work seeks to provide evidence-based insights that could help resolve the ongoing controversy over the health impacts of alkaline and acidic diets. By addressing the interplay between pH and antioxidant potential, this work seeks to provide insights that can inform dietary recommendations and promote the use of locally sourced vegetables as natural antioxidant sources in health and disease prevention.

2. Materials and Methods

2.1. Preparation of Samples

Vegetables were selected based on their availability and usage in Nigeria (Azeez et al., 2012; Iwalewa et al., 2005; Nwozo et al., 2015; Oboh, 2006; Ogunlade et al., 2012), colour-"red and green"- (Pennington & Fisher, 2009) and portion of the plant used for food –leaf, fruit, root/bulb (Lintas, 1992; Pennington & Fisher, 2009). Fresh vegetables -Amaranthus (*Amaranthus hybridus*), Spring Onion (*Allium fistulosum*), Ugwu (*Telfairia occidentalis*), Scent leaf (*Ocimum gratissimum*), Ewedu (*Corchorus olitorius*), Bitter leaf (*Vernonia amygdalina*) were purchased in the morning (between 6 and 7 am) from farmers in Ojo locality. Fresh Carrot (*Daucus carota*), Cabbage (*Brassica oleracea*), Cucumber (*Cucumis sativus)*, Sombo *(Capsicum frutescens)*, Tatashe (*Capsicum annuum*), Rodo (*Capsicum chinense*), Okro (*Abelmoschus esculentus*), Tomato (*Solanum lycopersicum*), Lettuce (*Lactuca sativa*) were bought very early in the morning (between 6 and 7am) from Iyana-iba market. The samples were immediately kept in a zip lock bag and placed on ice. Vegetable samples were washed with tap water after manually removing inedible parts. The vegetable samples were then sliced, blended (small amount of distilled water; less than 20mL to 100g was added to some of the samples to facilitate blending) and freeze-dried (Ilshin freeze dryer, Model no: FD5518). The collected samples were poured in plain bottles and stored in the refrigerator at 4°C until when ready for analysis

2.2. Determination of pH

A digital pH meter (Techmel and Techmel USA,Model: PHS-25) was used to determine acidity/alkalinity of both fresh and freeze-dried samples. Aliquots of fresh samples were used in the determination of the pH. The pH of freeze-dried samples was determined using a 10% solution of the freeze-dried samples (Tawo et al., 2009)

2.3. Determination of Phytochemical Constituent

2.3.1. Determination of Total Phenolic Compounds

A 0.5 mL of the extract (1mg/mL) was mixed with 1.5mL of 1:10 v/v Folin-Ciocalteu's reagent diluted with distilled water and allowed to stand for 25 °C for 5 min. Then 2mL of sodium carbonate (Na_2CO_3, 7.5 %, w/v) was added and the mixture was allowed to stand for another 90 min and kept in the dark with intermittent shaking. The absorbance of the colour formed was measured at 725 nm using a spectrophotometer. A standard curve was obtained using various concentrations (0-200 mg/mL) of freshly prepared Gallic acid solution. The results were expressed in mg/mL of the sample (Nwozo et al., 2015)

2.3.2. Determination of Ascorbic Acid Content

One milligram per mililitre concentration of samples was prepared in oxalic acid solution (0.5%). 10mL of each of the solutions were put in a test tube and 1 mL of $KMnO_4$ (100µg/mL in 5M H_2SO_4) was added. The solution was left to stand for 5 minutes. The absorbance was read at 530nm against blank (solution without sample). Standard calibration curve was obtained using various concentrations (100-1000µg/mL) of freshly prepared ascorbic (Elgailani et al., 2017).

2.4. Determination of Antioxidant Activity

2.4.1. Determination of 1,1-diphenyl-2- picryl-hydrazil (DPPH) Radical Scavenging Activity

A 2.0 mL aliquot of test sample (0, 50, 100, 200, 400, 800 and 1000µg/mL) was added to 2.0 mL of 0.16×10^{-3} mol/L DPPH ethanolic solution. The mixture was shaken for 1 min and then left to stand at room temperature for 30 min in the dark, and its absorbance measured using a Visible Pioway medical lab (V-5000) spectrophotometer at 517 nm. The ability to scavenge DPPH radical was calculated using the following equation:

DPPH radical scavenging activity (%) = [1 – (A sample – A sample blank)/A control] × 100

Where the A control is the absorbance of the control (DPPH solution without sample), the A sample is the absorbance of the test sample (DPPH solution plus test sample), and the A sample blank is the absorbance of the sample only (sample without DPPH solution). Ascorbic acid and BHT were used as reference compounds. IC_{50} was calculated as the concentration that scavenged 50% of the DPPH radicals (Thaipratum, 2014).

2.4.2. Determination of Ferric Reducing Antioxidant Power (FRAP) Assay

A 1.5mL of the different concentrations of plant extract was mixed with 2.5mL of 0.2M phosphate buffer (pH 6.6) and 1.5mL of 1% potassium hexacyanoferrate II. The mixture was incubated at 50°C for 20 minutes, 1.5mL of 10% TCA was added to the mixture and centrifuged at 3000 rpm for 10 minutes. The upper layer of the solution (1.5 mL) was mixed with distilled water (1.5mL) and $FeCl_3$ (0.3 mL, 0.1%), and the absorbance was measured using a Visible Pioway medical lab (V-5000) spectrophotometer at 700 nm. Ascorbic acid and BHT were used as reference compounds (Ak & Gülçin, 2008; Ganie et al., 2011) .

2.4.3. Determination of Nitric Oxide Radical (NO) Scavenging

This was measured according to the modified method of Badami et al.(2005). The reaction mixture (3 mL) containing 2 mL of 10 mM sodium nitroprusside, 0.5 mL of phosphate buffer saline (pH 7.4, 0.01M) and 0.5 mL of different concentrations of samples were incubated at 25°C for 150 min. Thereafter, 0.5mL of the reaction mixture containing nitrite was pipetted and mixed with 1 mL of sulphanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for complete diazotisation. Then, 1 mL of naphthylethylenediamine dihydrochloride (0.1%) was added, and allowed to stand for 30 min. The absorbance of the pink-coloured chromophore was measured using UV Visible Pioway medical lab (V-5000) spectrophotometer at 540nm against the corresponding blank solution.

% Inhibition = [(A0 – A1)/A0 x 100]

Where A0 is the absorbance of the control and A1 is the absorbance in the presence of the samples. The concentration providing 50% inhibition (IC_{50}) was calculated from the graph of percentage inhibition against sample concentrations (Badami et al., 2005).

2.4.4. Determination of Total Antioxidant Activity

Total antioxidant activity was determined according to the method of Rao et al. (2012). 1mL of the sample was combined with 3mL molybdenum reagent (50 mL of 0.6 M sulfuric acid, 50 mL of 28 mM sodium phosphate and 50 mL of 4 mM ammonium molybdate). The blank solution contained 4mL reagent solution only. The reaction mixture was incubated in an electric-heated thermostatic water bath (Model no: DKS 14) at 95 ºC for 90 min. After cooling to room temperature, the

absorbance was measured using a UV Visible Pioway medical lab (5100B) spectrophotometer at 695 nm. The total antioxidant activity of the sample was expressed as mg gallic acid equivalents (GAE)/g of extracts (Rao et al., 2012).

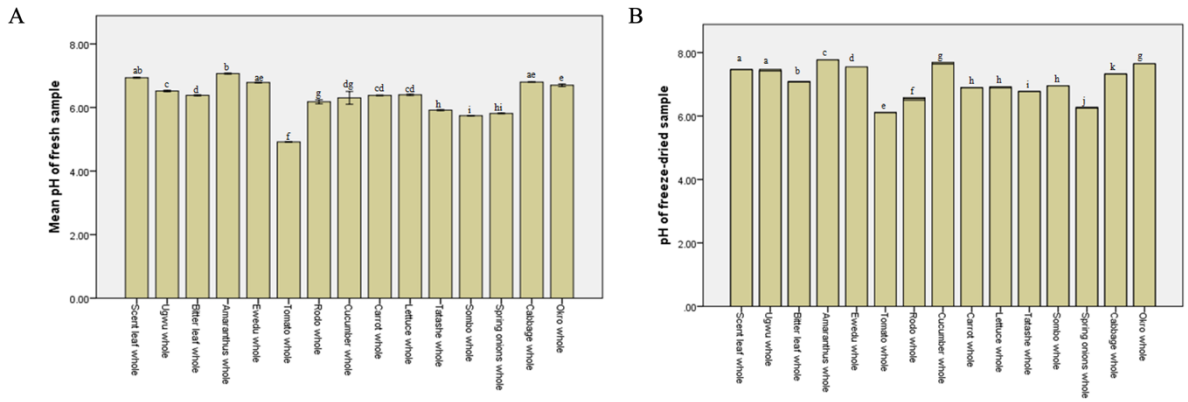
Roman typeface (e.g. Times, Times New Roman) throughout the paper. Font sizes and styles to be used in the paper are summarized in Table 3.

2.5. Statistical Analysis

The experiment was carried out in triplicates. The statistical package for social science (IBM- SPSS) software version 20 was used for data analysis. The results were expressed as mean± standard error. Analysis of variance (ANOVA) was used to compare means. Tukey's honestly significant difference (HSD) test was used for post hoc analysis. Spearman correlation analysis was used to determine the relationship between pH, phytochemical constituents, and antioxidant capacities of the vegetables. The analysis was conducted using R software (version 4.4.2). All results were expressed as mean ± standard deviation.

3. Results

The pH of the fresh vegetables (Figure 1a) ranged from 4.92 (Tomato) to 7.07 (Amaranthus). The pH of Amaranthus was significantly higher ($p< 0.05$) than other vegetables except Scent leaf. The pH of Tomato was significantly lower ($p< 0.05$) than other vegetables. The pH of freeze-dried vegetables (Figure 1b) ranged from 6.11 (Tomato) to 7.76 (Amaranthus). Just like the fresh samples, the pH of Amaranthus was significantly higher ($p< 0.05$) than other vegetables and that of tomato was significantly lower ($p< 0.05$) than other vegetables. The pH of freeze-dried samples followed the same pattern as that of the fresh samples but the pH of freeze-dried samples were relatively higher than the pH of fresh samples.



Figureure 1: pH of Vegetables. A. pH of fresh samples; B. pH of freeze-dried samples. Data is presented as Mean ± SD. [ab] Values with different superscripts are significantly different at $p < 0.05$

The Total phenolics concentration of vegetables (Figure 2a) ranged from 4.37mg/mL to 29.20mg/mL with Ugwu (29.20mg/mL) having the highest total phenolics concentration followed by Rodo (23.66mg/mL), Okro (23.66mg/mL), Tatashe (18.91mg/mL), Sombo (17.52mg/mL), Amaranthus (15.84mg/mL), Spring onion (12.36mg/mL), Ewedu (11.65mg/mL), Tomato (10.15 mg/mL) , Bitter leaf (8.97 mg/mL), Cucumber (6.25 mg/mL), Cabbage (4.60 mg/mL) and Carrot (4.37 mg/mL) having the lowest total phenolics concentration. The total phenolics concentration of Ugwu was significantly higher ($p< 0.05$) than other vegetables while that of carrot was significantly lower ($p< 0.05$) than other vegetables with the exception of Cabbage and Cucumber.

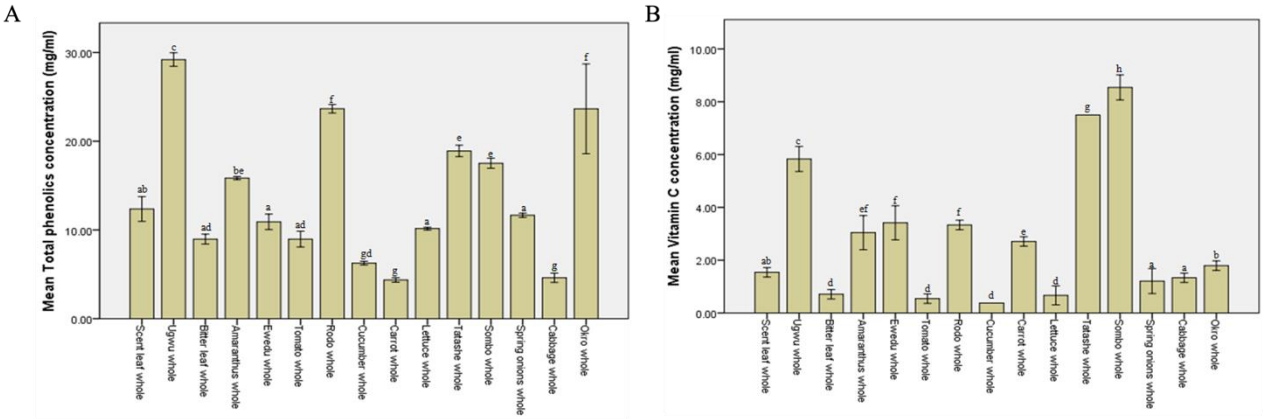


Figure 2: Phytochemical Analysis of Vegetables. A. Total phenolics content; B. Ascorbic acid content. Data is presented as Mean ± SD. [ab] Values with different superscripts are significantly different at $p < 0.05$

In decreasing order of concentration, ascorbic acid concentration (mg/mL) of the vegetables (Figure 2b) were Sombo (8.54mg/mL) > Tatashe (7.50 mg/mL) > Ugwu (5.83 mg/mL) > Ewedu (3.41mg/mL)

> Rodo (3.3mg/mL) > Amaranthus (3.04 mg/mL) > Carrot (2.71mg/mL) > Okro (1.79 mg/mL) > Scent leaf (1.54 mg/mL) > Cabbage (1.33 mg/mL) > Spring onion (1.21 mg/mL) > Bitter leaf (0.71 mg/mL) > Tomato (0.54 mg/mL) > Cucumber (0.38 mg/mL). Sombo had significantly higher ascorbic acid content ($p< 0.05$) compared to other vegetables and the ascorbic acid concentration of Cucumber was significantly lower ($p< 0.05$) when compared to other vegetables with the exception of Tomato, Lettuce and Bitter leaf.

From 400µg/mL, Ugwu (59.76%), Amaranthus (63.29%), Ewedu (59.29%), Tatashe (63.54%), Okro (62.00%) were comparable with BHT but at 1000µg/mL, Tatashe (98.87%) and Okro (95.34%) had higher DPPH radical Scavenging activity than Ascorbic acid (95.25%) (Figure 3a).

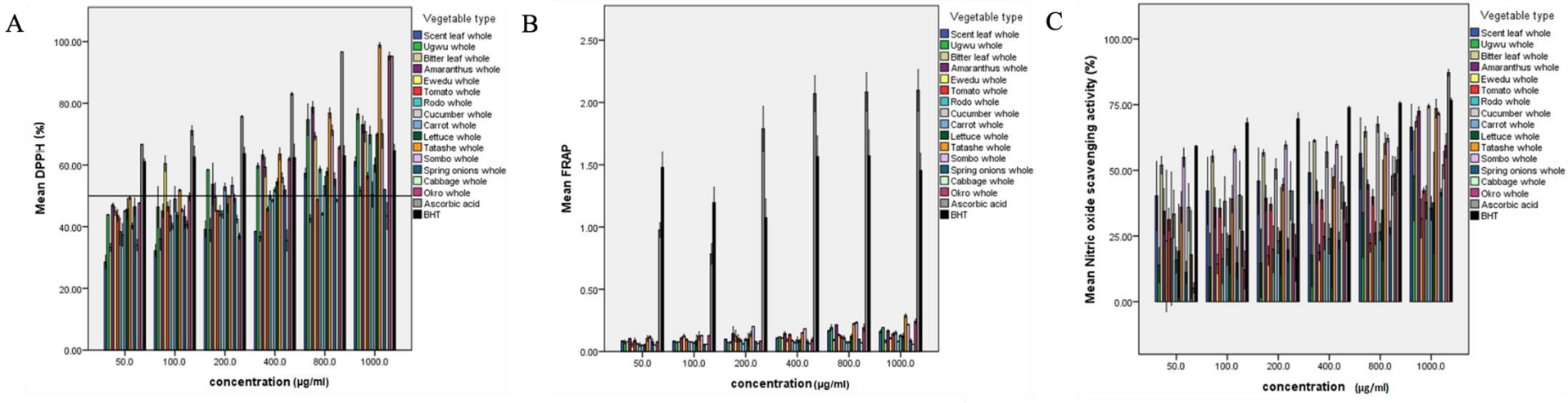
The Ferric reducing antioxidant potential of all the vegetables ((Figure 3b) was very low compared to ascorbic acid and BHT. Significant differences were observed from 200µg/mL for FRAP of all vegetables. The FRAP of all the vegetables ranged from 0.061 (Cabbage) to 0.18 (Sombo). All vegetables had a significantly low ($p< 0.05$) FRAP compared to the standards (Ascorbic acid and BHT). FRAP values for BHT and ascorbic acid were about 10 times higher than other vegetables.

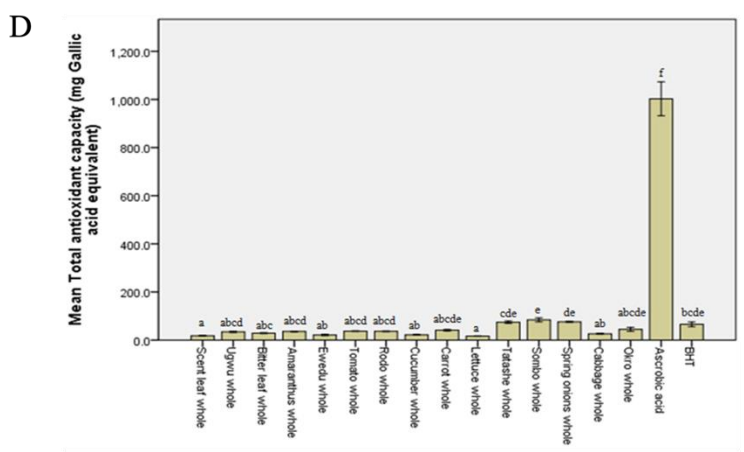
Nitric oxide scavenging activity (Figure 3c) of BHT (59.26%) was significantly higher ($p< 0.05$) than most of the vegetables and ascorbic acid (5.18%) from 50µg/mL; but ascorbic acid (53.95%) showed significant ($p< 0.05$) NO scavenging activity from 800µg/mL concentration. At 1000µg/mL the NO scavenging activity of ascorbic acid (87.21%) was higher than BHT (76.64%) and all other vegetables.

The total antioxidant capacity of vegetables (Figure 3d) ranged from 16.44mg Gallic acid equivalent (GAE) to 84.27mgGAE with Sombo having the highest total antioxidant capacity and Cucumber having the lowest. The total antioxidant capacity of all vegetables was significantly lower ($p<0.05$) compared to ascorbic acid (1002.778mgGAE) which is a standard antioxidant. The total antioxidant capacity of Tatashe (74.44mgGAE), Spring onions (75.94mgGAE) and Sombo (84.28mgGAE) were significantly higher ($p<0.05$) than BHT (65.6mgGAE).

The IC_{50} of DPPH radical scavenging activity (Figure 3e) ranged from 54.86 for Ewedu to1184.90 µg /mL for cabbage. The IC_{50} of vegetables were higher compared to standards: BHT (-2744 µg /mL) and ascorbic acid (-2933µg/mL). The IC_{50} of NO Scavenging activity (Figure 3e) of vegetables ranged from -443.1833µg/mL to 2124.69µg/mL with Sombo having the lowest IC_{50} value and carrot having the highest IC_{50} value. A negative IC_{50} value for Ascorbic acid and BHT may mean that the concentration of ascorbic acid and BHT used was very high.

Journal of Chemistry and Nutritional Biochemistry
https://jcnb.sabapub.com
E ISSN: 2709-5932





E

Vegetable type	DPPH IC_{50}	NO IC_{50}
Ascorbic acid	-2933.0000	405.44
BHT	-2744.0000	-1115.39
Ewedu whole	54.8600	2113.0533
Tatashe whole	119.2850	423.75
Ugwu whole	131.2400	1241.9667
Amaranthus whole	132.2200	584.15
Okro whole	228.8800	774.34
Sombo whole	238.0400	-443.18
Carrot whole	263.8000	2124.69
Lettuce whole	304.8900	1414.97
Rodo whole	391.3900	1511.75
Spring onions whole	466.6650	1471.97
Bitter leaf whole	586.8060	-216.74
Scent leaf whole	641.3000	403.66
Tomato whole	690.8550	1637.97
Cucumber whole	989.0950	314.57
Cabbage whole	1184.9000	781.70

Figure 3: Antioxidant Analysis of Vegetables. A. 1,1-diphenyl-2- picryl-hydrazil (DPPH) Radical Scavenging Activity; B. Ferric Reducing Antioxidant Power (FRAP) Assay; C. Nitric oxide (NO) scavenging activity; D. Total antioxidant capacity E. IC50 for DPPH and NO scavenging activity. Data is presented as Mean ± SD. [ab] Values with different superscripts are significantly different at $p < 0.05$.

There was significant positive correlation ($p<0.001$, $r=0.79$) between pH of fresh and freeze-dried samples (Figure 4). Significant positive correlation ($p<0.001$, $r= 0.66$) was observed between ascorbic acid concentration and total phenolics. Total antioxidant was also positively correlated ($p<0.001$, $r= 0.48$) with ascorbic acid concentration. Significant negative correlation was observed between IC_{50} of DPPH and total phenolic content ($p<0.001$, $r= -0.52$). Ascorbic acid was also negatively correlated ($p<0.001$, $r= -0.66$) with IC_{50} of DPPH. Significant negative correlation was seen between the pH of fresh ($p<0.001$, $r= -0.57$) and freeze-dried ($p<0.01$, $r= -0.40$) vegetables and their total antioxidant capacity. The IC_{50} of NO was also significantly negatively correlated with pH of freeze-dried samples.

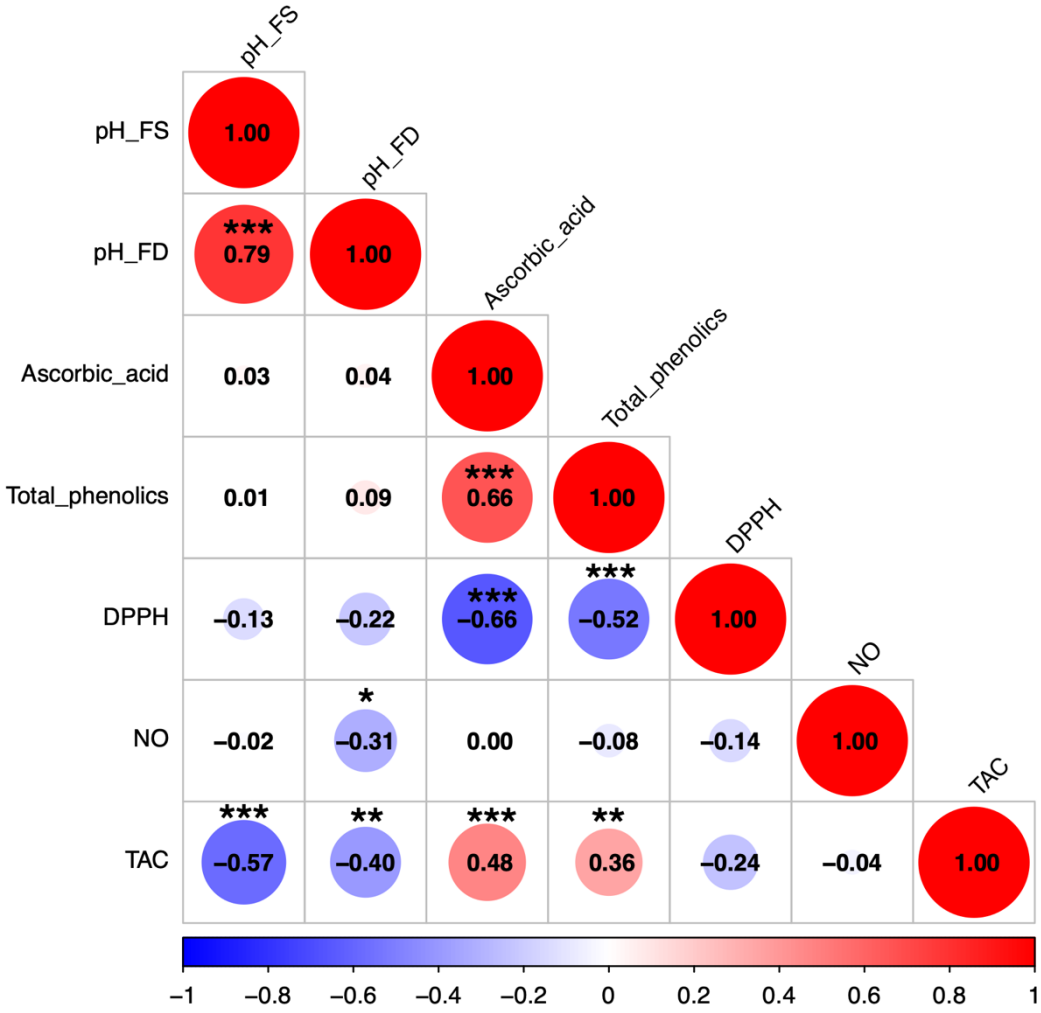


Figure 4: Correlation between pH, Phytochemical Constituents, and Antioxidant Capacities of Selected Vegetables. pH_FS denotes pH of fresh samples, pH_FD denotes pH of freeze-dried samples, Ascorbic_acid denotes Ascorbic acid concentration, Total_phenolics denotes Total phenolics concentration, DPPH denotes the 1,1-diphenyl-2- picryl-hydrazil (DPPH) Radical Scavenging Activity IC50, NO denotes the Nitric oxide scavenging activity IC50, TAC denotes the Total antioxidant capacity. Statistically, the data are represented as $p < 0.05$ for *, $p < 0.01$ for** and $p < 0.001$ for***.

4. Discussion

Hydrogen ion concentration (pH) is the measurement of the acidity or alkalinity of a solution commonly measured on a scale of 0 to 14. pH 7 is considered neutral, with lower pH values being acidic and higher values being alkaline (Baker & Silverton, 2014). Fruit and vegetable vary not only in the composition and quality of antioxidants, but also in natural acids. This suggests that fruit and vegetable have varying pH (Pękal & Pyrzynska, 2015). The freeze-drying process offers numerous advantages for the protection of natural products. With the removal of water, the taste, colour, texture and nutritional content of the food remains and a dried product with a characteristic aroma is obtained after freeze-drying. The rehydration process allows fruits to have almost the same quality as the fresh samples (George & Datta, 2002; Martínez-Romero et al., 2003).

The pH of the freeze-dried samples was relatively higher than those of the fresh samples. This could be due to the migration of water and dissolved substances within the material during drying inducing changes in pH, oxidation - reduction potential, and ionic strength (Lewicki, 1998). Similar observations were reported by Yurdugül & Bozoglu (2009) following their investigation of Vitamin C content and pH of fresh and freeze-dried plum samples. They found out that freeze drying did not decrease the Vitamin C content but caused a slight increase in the pH of plum samples (Yurdugül & Bozoglu, 2009) further supporting the result of this study.

Typical compounds that possess antioxidant activity include vitamin C, vitamin E, carotenoids, and phenolic compounds. It was therefore reasonable to investigate vitamin C and total phenolic content to identify the phytochemicals that most significantly contribute to the antioxidant capacity of the selected vegetables (Chanwitheesuk et al., 2005). Phenolic compounds in plants are powerful free radical scavengers that can inhibit lipid peroxidation by neutralizing peroxyl radicals generated during the oxidation of lipids (Shahidi et al., 1992) . Their antioxidant capacity is influenced by the number and position of hydroxyl groups in the ring structure (Olszowy, 2019). Most phenolics found in vegetables are water soluble in nature (Edet et al., 2015). The study of Bayili et al. (2011) on the HCl/Methanol (1%v/v) extract of some vegetables in Burkina Faso which includes cabbage, cucumber, sombo, tomato and rodo reported that *sombo* had the highest total phenolic content (333.5 mg GAE/100 g). This finding aligns closely with the results of this study. Similarly, Edet et al. (2015) found that the phenolic content of water extracts from *ugwu* (137.67 mg GAE/100 g) exceeded that of bitter leaf (97.33 mg GAE/100 g) in a study conducted in Nigeria, which is similar to what was observed in our study.

Vitamin C is an essential water-soluble antioxidant needed by the human body. Ascorbic acid is of great importance in biochemical reactions as a reducing agent (Edet et al., 2015; Fadhel, 2012) It is one of the most important vitamins in fruits and vegetables. Unlike most animals, humans and other primates cannot synthesise their vitamin C (Elgailani et al., 2017). A study by Oboh and Akindahunsi (2004) reported the ascorbic acid content of five vegetables (Ugwu, Scent leaf, Amaranthus, Ewedu

and Bitter leaf) used in this study. In their study, Ugwu had higher ascorbic acid content than other vegetables as was also seen in our study (Oboh & Akindahunsi, 2004).

The efficacy of antioxidants is dependent on the type of oxidants. For example, vitamin E (α-tocopherol) is a potent radical-scavenging antioxidant but less effective against lipid peroxidation by lipoxygenase. Conversely, carotenoids may exhibit weak radical scavenging activity but are potent inhibitors of oxidation induced by singlet oxygen. Oxidative damage can be attenuated not only by scavenging radicals but also by sequestering metal ions, decomposing hydrogen peroxide and/or hydroperoxides, quenching active prooxidants, and repairing damage. An antioxidant can exert its effect by different mechanisms and functions and it is essential in evaluating antioxidant capacity to clarify which function is being measured by the method employed. Thus, as often pointed out, no single method correctly evaluates the total antioxidant capacity. As such, methods should be selected considering the function to be evaluated (Niki & Noguchi, 2000). In this study, different methods were used for the evaluation of antioxidant activity *in vitro.*

The DPPH radical scavenging assay is one of the most commonly used methods to evaluate the radical scavenging activity of antioxidants because of its quickness, reliability, and reproducibility. This method depends on the ability of compounds to reduce the purple DPPH by accepting an electron or hydrogen radical to become a stable diamagnetic molecule with discolouration. The degree of discolouration indicates the free radical scavenging potentials of the antioxidant compounds or extracts (Kedare & Singh, 2011; Mishra et al., 2012; Molyneux, 2004; Shahidi et al., 1992). All tested concentrations showed a promising DPPH scavenging effect, with Tatashe and Okro exhibiting higher activity than Ascorbic acid and BHT. Okro and Tatashe also had high phenolic and ascorbic acid contents which may be responsible for their high DPPH scavenging activity (Tavarini et al., 2008).

FRAP assay is based on the reduction of Fe^{3+}/ferricyanide complex to its ferrous form which can then be monitored spectrophotometrically at 700 nm. Increased absorbance of the reaction mixture indicates increased reducing power (Ak & Gülçin, 2008; Ganie et al., 2011). FRAP of Rodo, Tatashe and Sombo determined by Ogunlade *et al.* (2012) showed that Rodo had a higher FRAP than Tatashe and Sombo which also agrees with the result of this study. Generally, the FRAP was very low compared to Ascorbic acid. This could mean that the antioxidant activity of the vegetables may be more of radical scavenging than reduction (Ogunlade et al., 2012)

Sodium nitroprusside solution at physiological pH, spontaneously produces nitric oxide, which reacts with oxygen to produce nitrite ions according to the Griess Illosvoy reaction. Nitric oxide or reactive nitrogen species, formed during their reaction with oxygen or superoxides are very reactive. These compounds/radicals are responsible for the changes in the structural and functional responses of many cellular components, causing serious toxic reactions with proteins, lipids, nucleic acids among others (Lawal et al., 2015). The NO scavenging activities of the vegetables studied were high across all concentrations showing promising NO scavenging activity.

The total antioxidant capacity of the extracts was measured spectrophotometrically through phosphomolybdenum method, based on the reduction of Mo (VI) to Mo (V) by the test sample and the subsequent formation of green phosphate-Mo (V) compounds (Vijayakumar et al., 2013). The total antioxidant activity of vegetables was low compared to ascorbic acid. This indicates that the antioxidant activity exhibited by the vegetables could be due to radical scavenging than reduction.

Sombo, Tatashe and spring onion which had high phenolics concentration also had higher total antioxidant activity, compared to other vegetables. Recent studies have shown that many polyphenols contribute significantly to the phosphomolybdate scavenging activity of medicinal plants (Chaudhary et al., 2020; Khiya et al., 2021)

The IC_{50} values (the concentration required to inhibit 50% of radicals), were calculated to compare antioxidant efficacy. The lower the IC_{50} value, the greater the free radical scavenging activity (Ganie et al., 2010). The highest DPPH radical scavenging effect was obtained in ewedu with the lowest DPPH IC_{50} value followed by tatashe, ugwu and amaranthus. sombo, tatashe, scent leaf and cucumber had lower NO IC_{50} values compared to ascorbic acid. Vegetables with high ascorbic acid and phenolic contents as reported in this study also had increased capability of donating hydrogen atoms to scavenge free radicals.

Since there are different types of antioxidant compounds which contribute to the total antioxidant capacity, it is not clear which components are responsible for the observed antioxidant capacity. To explore the influence of the phytochemical constituents on antioxidant capacity we determined the correlation between the antioxidant capacity and main phytochemical constituents (vitamin C and total phenols). The antioxidant capacities of the selected vegetables appear to be largely influenced by vitamin C and phenol levels (Du *et al.*, 2009). In our study, vitamin C content was strongly correlated with antioxidant capacity (Total antioxidant and DPPH). The positive correlation between total antioxidant capacity and ascorbic acid concentration implies that vitamin C was a major contributor to the total antioxidant capacity in the selected vegetables. Tavarini et al. (2008) also reported that ascorbic acid was the major contributor to total antioxidant capacity of Kiwi fruits.

Ascorbic acid and phenolic contents of fruits and vegetables have also been linked to their DPPH radical scavenging activity (Dumbravă et al., 2011; Souri et al., 2008; Tiveron et al., 2012; Kevers et al., 2007). The negative correlation between vitamin C concentration and IC_{50} of DPPH also buttressed this assertion. Total phenolics and vitamin C concentration were also positively correlated indicating that vegetables with high ascorbic acid content also had high phenolic content. This may imply that depending on the method of antioxidant assay used both ascorbic acid and total phenols contributed greatly to the antioxidant capacity of the vegetables (Du et al., 2009).

Several studies have reported on the relationships between phenolic content and antioxidant activity. Some authors found a correlation between the phenolic content and the antioxidant activity, while others found no such relationship (Ismail et al., 2004). No correlation between antioxidant activity and phenolic content was found in the study by Ismail et al. (2004) on some plant extracts containing

phenolic compounds. In their study, cabbage had the lowest total phenolic content whereas its antioxidant activity was a bit higher than that of kale which had a higher total phenolic content. On the contrary, Velioglu et al. (1998) and Gardner et al. (2000) reported a strong relationship between total phenolic content and antioxidant activity in selected fruits, vegetables and grain products (Gardner et al., 2000; Velioglu et al., 1998). Contradictions in the relationship between total phenolic content and antioxidant activity may be a result of a wide degree of variation between different phenolic compounds in their effectiveness as antioxidants (Robards et al., 1999). In our study, we observed significant negative correlation ($p<0.05$) between IC_{50} DPPH and total phenolic content indicating that as total phenolic content increased the DPPH radical scavenging activity also increased. The significant negative correlation ($p<0.001$) between the pH of both fresh and freeze-dried samples and total antioxidant capacity implies that as the pH increased the antioxidant capacity reduced. Adu et al. (2018) had earlier reported a negative correlation between pH and antioxidant capacities (DPPH and FRAP). Their result showed that antioxidant capacities increased with increasing acidity which supports the findings of this study. Similarly, Kalt et al. (2000) reported that the antioxidant capacity measured via the oxygen radical absorbance capacity (ORAC) in blueberry juice increased with higher acidity (pH 1–4) (Kalt et al., 2000). This higher antioxidant capacity was attributed to the abundance of phenolic compounds and anthocyanins in the more acidic samples. However, in our study, no direct correlation between pH, phenolic and ascorbic acid content was observed which may be due to the diversity of samples analyzed and inherent variations among them. We also see in our study the moderately significant negative correlation ($p<0.01$) between the pH of freeze-dried samples and the NO IC_{50}. (Bang-Weon (1987) reported that the nitrite scavenging activity of their tested vegetables including carrot was pH dependent with the highest activity at pH 1.2 and lowest at pH 6.0 (Bang-Weon, 1987). This shows that the NO scavenging activity increased as acidity increased which is similar to the findings of this study. Altogether, these findings negate the popular view that alkaline foods have higher antioxidant capacity (Remer & Manz, 1995; Suthar & Verma, 2014).

5. Conclusion

Results of this study revealed that all the fresh vegetables used in this study, except Amaranthus, had a pH less than 7 but freeze-drying these vegetables increased their pH. Also, the vegetables with higher concentration of phytochemicals- ascorbic acid and total phenolic- have higher antioxidant capacities. This aligns with broader research indicating that the antioxidant capacity of vegetables is influenced by multiple phytochemical constituents. In addition, the antioxidant potential of these vegetables increased with increasing acidity which is contrary to popular belief.

Author Contributions

Adu O.B conceptualized the idea, Adu O.B, Saibu G.M. and Ogun S.O designed the analysis. Adu O.B and Saibu G.M. supervised the work. Ogun S.O and Akanni M.H performed the experiment. Adu O.B and Ogun S.O processed the experimental data and performed the data analysis. Ogun S.O interpreted the results and wrote the manuscript with input from all authors.

References

Ak, T., & Gülçin, I. (2008). Antioxidant and radical scavenging properties of curcumin. *Chemico-Biological Interactions*, *174*(1), 27–37.

Akbarirad, H., Ardabili, A. G., Kazemeini, S. M., & Khaneghah, A. M. (2016). An overview on some of important sources of natural antioxidants. *International Food Research Journal*, *23*(3).

Amarowicz, R., Pegg, R. B., Rahimi-Moghaddam, P., Barl, B., & Weil, J. A. (2004). Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chemistry*, *84*(4), 551–562.

Azeez, L., Adeoye, M. D., Majolagbe, T. A., Lawal, A. T., & Badiru, R. (2012). Antioxidant activity and phytochemical contents of some selected Nigerian fruits and vegetables. *American Journal of Chemistry*, *2*(4), 209–213.

Badami, S., Rai, S. R., & Suresh, B. (2005). Antioxidant activity of Aporosa lindleyana root. *Journal of Ethnopharmacology*, *101*(1–3), 180–184.

Baker, F. J., & Silverton, R. E. (2014). *Introduction to medical laboratory technology*. Butterworth-Heinemann.

Bang-Weon, A. H. N. (1987). Degradation of Carcinogenic Nitrosamine Formation Factor by Natural Food Components 1. Nitrite-scavenging Effects of Vegetable Extracts. *Korean Journal of Fisheries and Aquatic Sciences*, *20*(5), 463–468.

Bayili, R. G., Abdoul-Latif, F., Kone, O. H., Diao, M., Bassole, I. H. N., & Dicko, M. H. (2011). Phenolic compounds and antioxidant activities in some fruits and vegetables from Burkina Faso. *African Journal of Biotechnology*, *10*(62), 13543–13547.

Birt, D. F., Hendrich, S., & Wang, W. (2001). Dietary agents in cancer prevention: flavonoids and isoflavonoids. *Pharmacology & Therapeutics*, *90*(2–3), 157–177.

Chanwitheesuk, A., Teerawutgulrag, A., & Rakariyatham, N. (2005). Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand. *Food Chemistry*, *92*(3), 491–497.

Dragsted, L. O., Pedersen, A., Hermetter, A., Basu, S., Hansen, M., Haren, G. R., Kall, M., Breinholt, V., Castenmiller, J. J. M., & Stagsted, J. (2004). The 6-a-day study: effects of fruit and vegetables on markers of oxidative stress and antioxidative defense in healthy nonsmokers. *The American Journal of Clinical Nutrition*, *79*(6), 1060–1072.

Du, G., Li, M., Ma, F., & Liang, D. (2009). Antioxidant capacity and the relationship with polyphenol and vitamin C in Actinidia fruits. *Food Chemistry*, *113*(2), 557–562.

Dumbravă, D.-G., Hădărugă, N.-G., Moldovan, C., Raba, D.-N., Popa, M.-V., & Rădoi, B. (2011). Antioxidant activity of some fresh vegetables and fruits juices. *Journal of Agroalimentary Processes and Technologies*, *17*(2), 163–168.

Edet, E. E., Ofem, J. E., Igile, G. O., Ofem, O. E., Zainab, D. B., & Akwaowo, G. (2015). Antioxidant capacity of different African seeds and vegetables and correlation with the contents of ascorbic acid, phenolics and flavonoids. *Journal of Medicinal Plants Research*, *9*(13), 454–461.

Elgailani, I. E. H., Elkareem, M., Noh, E., Adam, O., & Alghamdi, A. (2017). Comparison of two methods for the determination of vitamin C (ascorbic acid) in some fruits. *Am. J. Chem*, *2*(1), 1–7.

Fadhel, D. H. (2012). Spectrophotometric determination of ascorbic acid in aqueous solutions. *Al-Nahrain Journal of Science*, *15*(3), 88–94.

Felter, S. P., Zhang, X., & Thompson, C. (2021). Butylated hydroxyanisole: Carcinogenic food additive to be avoided or harmless antioxidant important to protect food supply? *Regulatory*

Toxicology and Pharmacology, *121*, 104887. https://doi.org/https://doi.org/10.1016/j.yrtph.2021.104887

Ganie, S. A., Haq, E., Masood, A., Hamid, A., & Zargar, M. A. (2011). Antioxidant and protective effect of ethyl acetate extract of podophyllum hexandrum rhizome on carbon tetrachloride induced rat liver injury. *Evidence-Based Complementary and Alternative Medicine*, *2011*(1), 238020.

Ganie, S. A., Zargar, M. A., Masood, A., & Haq, E. (2010). In vitro and in vivo evaluation of free radical scavenging potential of ethanolic extract of Podophyllum hexandrum. *Afr J Biochem Res*, *4*(7), 191–195.

Gardner, P. T., White, T. A. C., McPhail, D. B., & Duthie, G. G. (2000). The relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. *Food Chemistry*, *68*(4), 471–474.

George, J. P., & Datta, A. K. (2002). Development and validation of heat and mass transfer models for freeze-drying of vegetable slices. *Journal of Food Engineering*, *52*(1), 89–93.

Gobbo-Neto, L., & Lopes, N. P. (2007). Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. *Química Nova*, *30*, 374–381.

Gulcin, İ. (2020). Antioxidants and antioxidant methods: an updated overview. *Archives of Toxicology*, *94*(3), 651–715. https://doi.org/10.1007/s00204-020-02689-3

Ismail, A., Marjan, Z. M., & Foong, C. W. (2004). Total antioxidant activity and phenolic content in selected vegetables. *Food Chemistry*, *87*(4), 581–586.

Iwalewa, E. O., Adewunmi, C. O., Omisore, N. O. A., Adebanji, O. A., Azike, C. K., Adigun, A. O., Adesina, O. A., & Olowoyo, O. G. (2005). Pro-and antioxidant effects and cytoprotective potentials of nine edible vegetables in southwest Nigeria. *Journal of Medicinal Food*, *8*(4), 539–544.

Kalt, W., McDonald, J. E., & Donner, H. (2000). Anthocyanins, phenolics, and antioxidant capacity of processed lowbush blueberry products. *Journal of Food Science*, *65*(3), 390–393.

Kedare, S. B., & Singh, R. P. (2011). Genesis and development of DPPH method of antioxidant assay. *Journal of Food Science and Technology*, *48*, 412–422.

Lawal, O. A., Ojekale, A. B., Oladimeji, O. S., Osinaike, T. S., Sanni, A. A., Simelane, M. B. C., Mosa, R. A., & Opoku, A. R. (2015). In vitro antioxidant activity, total phenolic and total flavonoid contents of essential oils of three Cyperus species (Cyperaceae). *Br J Pharm Res*, *7*(1), 52–62.

Lewicki, P. P. (1998). Effect of pre-drying treatment, drying and rehydration on plant tissue properties: A review. *International Journal of Food Properties*, *1*(1), 1–22.

Lintas, C. (1992). Nutritional aspects of fruits and vegetables consumption. *Options Mediterraennes*, *19*, 79–87.

Martínez-Romero, D., Castillo, S., & Valero, D. (2003). Forced-air cooling applied before fruit handling to prevent mechanical damage of plums (Prunus salicina Lindl.). *Postharvest Biology and Technology*, *28*(1), 135–142.

Mishra, K., Ojha, H., & Chaudhury, N. K. (2012). Estimation of antiradical properties of antioxidants using DPPH assay: A critical review and results. *Food Chemistry*, *130*(4), 1036–1043.

Mitterer-Daltoe, M., Bordim, J., Lise, C., Breda, L., Casagrande, M., & Lima, V. (2020). Consumer awareness of food antioxidants. Synthetic vs. Natural. *Food Science and Technology*.

Mohammed, F. H., Al-Saily, H. M. N., & Awadh, E. F. A. (2023). Using Alkaline Diet for Controlling Blood Acidosis. *Journal of University of Babylon for Pure and Applied Sciences*, 312–321.

Molyneux, P. (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J. Sci. Technol*, *26*(2), 211–219.

Niki, E., & Noguchi, N. (2000). *Evaluation of antioxidant capacity. What capacity is being measured by which method?*

Nwozo, S. O., Oso, B. J., & Oyinloye, B. E. (2015). Effect of heat on antioxidant activity of some tropical leafy vegetables. *Nigerian Journal of Basic and Applied Sciences*, *23*(2), 93–101.

Oboh, G. (2006). Nutritive value, antioxidant and antimicrobial properties of Struchium sparganophora leaves. *Journal of Medicinal Food*, *9*(2), 276–280.

Oboh, G., & Akindahunsi, A. A. (2004). Change in the ascorbic acid, total phenol and antioxidant activity of sun-dried commonly consumed green leafy vegetables in Nigeria. *Nutrition and Health*, *18*(1), 29–36.

Ogunlade, I., Alebiosu, A. A., & Osasona, A. I. (2012). Proximate, mineral composition, antioxidant activity, and total phenolic content of some pepper varieties (Capsicum species). *International Journal of Biological and Chemical Sciences*, *6*(5), 2221–2227.

Olszowy, M. (2019). What is responsible for antioxidant properties of polyphenolic compounds from plants? *Plant Physiology and Biochemistry*, *144*, 135–143. https://doi.org/https://doi.org/10.1016/j.plaphy.2019.09.039

Passey, C. (2017). Reducing the Dietary Acid Load: How a More Alkaline Diet Benefits Patients With Chronic Kidney Disease. *Journal of Renal Nutrition*, *27*(3), 151–160. https://doi.org/https://doi.org/10.1053/j.jrn.2016.11.006

Pękal, A., & Pyrzynska, K. (2015). Effect of pH and metal ions on DPPH radical scavenging activity of tea. *International Journal of Food Sciences and Nutrition*, *66*(1), 58–62.

Pennington, J. A. T., & Fisher, R. A. (2009). Classification of fruits and vegetables. *Journal of Food Composition and Analysis*, *22*, S23–S31.

Poprac, P., Jomova, K., Simunkova, M., Kollar, V., Rhodes, C. J., & Valko, M. (2017). Targeting free radicals in oxidative stress-related human diseases. *Trends in Pharmacological Sciences*, *38*(7), 592–607.

Rao, K. S., Keshar, N. K., & Kumar, B. R. (2012). A comparative study of polyphenolic composition and in-vitro antioxidant activity of Illicium verum extracted by microwave and soxhlet extraction techniques. *Ind J Pharm Edu Res*, *46*(3), 228.

Remer, T., & Manz, F. (1995). Potential Renal Acid Load of Foods and its Influence on Urine pH. *Journal of the American Dietetic Association*, *95*(7), 791–797. https://doi.org/https://doi.org/10.1016/S0002-8223(95)00219-7

Robards, K., Prenzler, P. D., Tucker, G., Swatsitang, P., & Glover, W. (1999). Phenolic compounds and their role in oxidative processes in fruits. *Food Chemistry*, *66*(4), 401–436.

Shahidi, F., Janitha, P. K., & Wanasundara, P. D. (1992). Phenolic antioxidants. *Critical Reviews in Food Science & Nutrition*, *32*(1), 67–103.

Souri, E., Amin, G., Farsam, H., & Barazandeh, T. M. (2008). *Screening of antioxidant activity and phenolic content of 24 medicinal plant extracts*.

Suthar, N. N., & Verma, A. P. (2014). ALKALINE DIET AND HEALTH-A BRIEF REVIEW. *International Journal of Basic & Applied Physiology*, *3*(1).

Tavarini, S., Degl'Innocenti, E., Remorini, D., Massai, R., & Guidi, L. (2008). Antioxidant capacity, ascorbic acid, total phenols and carotenoids changes during harvest and after storage of Hayward kiwifruit. *Food Chemistry*, *107*(1), 282–288.

Tawo, E. N., Abara, A. E., Malu, S. P., & Alobi, N. O. (2009). Evaluation of pH levels in some common carbohydrate food items consumed by communities in the Central Senatorial District of Cross River State, South-South of Nigeria. *Pakistan Journal of Nutrition*, *8*(9), 1387–1390.

Thaipratum, R. (2014). Evaluation of antioxidant activities of cabbage (Brassica oleracea L. var. capitata L.). *International Journal of Bioengineering and Life Sciences*, *8*(6), 591–593.

Tiveron, A. P., Melo, P. S., Bergamaschi, K. B., Vieira, T. M. F. S., Regitano-d'Arce, M. A. B., & Alencar, S. M. (2012). Antioxidant activity of Brazilian vegetables and its relation with phenolic composition. *International Journal of Molecular Sciences*, *13*(7), 8943–8957.

Tucker, K. L., Hannan, M. T., & Kiel, D. P. (2001). The acid-base hypothesis: diet and bone in the Framingham Osteoporosis Study. *European Journal of Nutrition*, *40*, 231–237.

Velioglu, Y., Mazza, G., Gao, L., & Oomah, B. D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural and Food Chemistry*, *46*(10), 4113–4117.

Venkatachalam, K., Rangasamy, R., & Krishnan, V. (2014). Total antioxidant activity and radical scavenging capacity of selected fruits and vegetables from South India. *International Food Research Journal*, *21*(3).

Vijayakumar, A., Kumar, P. P., & Jeyaraj, B. (2013). Antioxidant activity of Illicium griffithi hook. F. & thoms seeds-in vitro. *Asian Journal of Pharmaceutical and Clinical Research*, *6*(2), 269–273.

Yurdugül, S., & Bozoglu, F. (2009). *Studies on antimicrobial activity and certain chemical parameters of freeze-dried wild plums (Prunus spp.).*

Zhu, L., Luo, M., Zhang, Y., Fang, F., Li, M., An, F., Zhao, D., & Zhang, J. (2023). Free radical as a double-edged sword in disease: Deriving strategic opportunities for nanotherapeutics. *Coordination Chemistry Reviews*, *475*, 214875.