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The Effects of Different Concentrations of Sprouted And Boiled Onions Extracts On the Antioxidant Potentials Of Onions (*Allium cepa* L.)

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Abstract
After sprouting of onions, the shoots are used as vegetables and bulbs discarded. These usually discarded onion bulbs may have improved antioxidant potentials resulting from sprouting. These improved properties could be harnessed to combat or manage some degenerative and non-communicable diseases. This study was therefore conducted to determine the effects of sprouting and boiling on the antioxidant potentials of onions (Allium cepa L.). Samples were either sprouted for 0 – 10 days or boiled for 0 – 8 minutes. Phytochemical (total phenols, flavonoids, ascorbic acid) analyses and antioxidant activities such as reducing power, DPPH and ABTS radicals scavenging activities were used to assess antioxidant potentials using standard methods.The results show that regardless of the nature of solvent, boiling resulted in significant reduction in total phenols ($P<0.05$). The least reduction (36%) was observed in the chloroform extract of samples boiled for 8min. Aqueous and methanol extracts recorded 42% reductions. A significantly ($P<0.05$) higher total flavonoid content was expressed in methanol extract of onions sprouted for eight days (7.84mg/g RE). The reducing potential was found to be concentration dependent up to 0.4 mg/ml. From the EC_{50} values, the strongest reducing power was exhibited by the extract from onion bulbs sprouted for 6 days while the weakest reducing power was noticed with day 8 (EC_{50} = 0.73) and day 10 (EC_{50} = 0.74). This trend follows the same pattern with ascorbic acid content. In DPPH and ABTS, the strongest radical scavenging capacity was exhibited by the extract from onion bulbs sprouted for 4 days (EC_{50} = 0.01) and (EC_{50} = 0.002) respectively. Generally, sprouting for 2 – 8 days resulted in a significant ($P<0.05$) increase in all the antioxidant parameters tested. This was followed by a slight but significant decrease at the 10^{th} day of sprouting. Boiling for up to 8 minutes resulted in significant losses in all antioxidant parameters tested. The present study shows that sprouted onions demonstrated higher antioxidant activity and can be considered as good sources of natural antioxidants.

Keywords: *Onions, Antioxidant, Sprouting, Boiling, Methanol, Chloroform, DPPH and ABTS*

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I. Introduction

Processing methods are known to have variable effects on total phenolic compound and antioxidant activity of plant samples. Effects include little or no change, significant losses, or enhancement in antioxidant activity (Nicoli *et al*., 1999). Food processing can improve the properties of naturally occurring antioxidants or induce the formation of new compounds with antioxidant activity, so that the overall antioxidant activity increases or remains unchanged (Tomaino *et al*., 2005).

Antioxidants present in vegetables are very useful and beneficial to health and have been associated with reduced risk of cardiovascular diseases and various forms of cancer (Kumud *et al*., 1990). These benefits have led to research studies in order to find antioxidants in plant material mainly used as foods (Yang *et al*., 2008). Among the compounds with antioxidant properties are the phenolics, which are believed to act as antioxidant, anti-carcinogenic, anti-microbial, anti-allergic, anti-mutagenic and anti-inflammatory, as well as in the reduction of cardiovascular diseases (Vali *et al*., 2007). Phenolics occur naturally in plants and are present in fruits, vegetables, leaves, nuts, seeds and flowers; therefore, they are present in the human diet, but are also used in some medicinal preparations (Madrau *et al*., 2008).

Onions *(Allium cepa* Linn) is used as foodstuff, condiments, flavouring agent, and in folk medicine (Ola-Mudathir and Maduagwu, 2014). It has been extensively studied for their therapeutic uses as antibiotic, antidiabetic, anti-atherogenic and anticancer (Augusti, 1996). It has been found that administration of onion products to diabetic rats significantly reduced hyperglycaemia (Kumud *et al*., 1990). Biological action of *Allium*

products is ascribed to organosulfur compounds, which have also been shown to possess antioxidant and free radical scavenging activities. Onions have previously been shown to protect testis against cadmium induced oxidative stress in rats (Ola-Mudathir *et al*., 2008). Keeping this in mind, many studies have reported losses in total phenolic content and antioxidant activity of plant samples following thermal treatments. Losses were mainly reported in vegetables (Ismail *et al*., 2004; Roy *et al*., 2007; Toor and Savage, 2006). These losses in antioxidant property of heat-treated samples were attributed to thermal degradation of phenolic compounds (Larrauri *et al*., 1997) as well as other methods of food processing. However, there still remains paucity of information on the effect of different processing methods on the antioxidant status of onions which is essentially used in most kitchens for the preparation of delicacies as well as in the preparation of decoctions used by trado-medical practitioners for treatment of some ailments. There is however a few reported studies on the effect of domestic processing on the antioxidant potentials of onions. Such information would be more relevant considering the fact that onions are rarely consumed raw without processing. Common processing methods include: remover of dry skin, chopping into smaller pieces before boiling, grilling or flying in oil. Several cultures also subject onions to sprouting for the purpose of using the shoots as vegetables. After sprouting, the onions bulbs are usually discarded while the shoots are processed further. These usually discarded onion bulbs may have improved antioxidant potentials resulting from sprouting. These improved properties could be harnessed to combat or manage some degenerative and non-communicable diseases.

II. Materials And Methods

Collection and Preparation of Sample

Sample of mature onions *(Allium cepa L.*) were obtained from a local Market in Esan West Local Government Area (Ekpoma) in Edo State. The dry skin of onions was removed and placed on stainless tray already over laid with wet tissue paper. They were allowed to sprout for up to 10 days in the dark at 25 – 30°C. Separately, slices of the onion sample were also boiled for 2 – 8 minutes (mins) respectively while un-boiled/un-sprouted onion served as control for both. Subsequently 25g of the raw and the processed samples were minced, grounded and extracted with different solvents (water, methanol and chloroform).

Method of Extraction

About 25 g of the sprouted onions (0 day, 2 days, 4 days, 6 days, 8 days and 10 days) and boiled onions (0 min, 2 mins, 4 mins, 6 mins, and 8 mins) were separately weighed using an analytical chemical balance into different beakers. They were homogenized separately using a laboratory mortar and pestle. The homogenized sample was then extracted with 100ml of three different solvents: methanol, chloroform and water. The samples were centrifuged at 4000 rpm for 30 minutes. The supernatant was filtered through a filter paper (Whatman No. 1) into a beaker after which, it was concentrated and dissolved in dimethyl sulphoxide (DMSO).

Determination of Total Phenolic Content (TPC) of *Allium cepa* Extract

The concentration of phenolic compounds in the *Allium cepa* L. (sprouted and boiled) extracts were expressed as pyrocatechol equivalents (PEs) determined by Folin-Ciocalteu reagent according to the method of Slinkard and Singleton (1997) with minor modification. Briefly, 1 ml of the onion extract was measured into a volumetric flask and was filled with 46 ml of distilled aqueous. Briefly 1 ml of Folin-Ciocalteu reagent was added and mixed thoroughly. After 3 minutes, 3 ml of 2% anhydrous sodium carbonate (Na_2CO_3) was added and then allowed to stand for 2 hours with intermittent shaking. The absorbance was measured at 760 nm in a spectrophotometer (JENWAY 6715, Bibby Scientific Ltd UK) against a blank consisting of all the reaction agents except the extracts.

The total concentration of phenolic compounds in the extract was determined as microgram of pyroctechol equivalent by using an equation that was obtained from standard pyrocatechol graph as:

$$\text{Absorbance} = 0.0021 \times \textit{total phenols}\ (\mu\text{gpyrocatechol}) - 0.0092 \quad (R^2 = 0.9934)$$

Determination of Total Flavonoid Content of *Allium cepa* L. Extract

The total flavonoid was determined using the method of Meda *et al*. (2005). Briefly, 2ml of 2% aluminium trichloride ($AlCl_3$) in methanol was mixed with the same volume of the extract solution. The mixture was incubated at room temperature for 10 minutes, and the absorbance was measured at 415nm in spectrophotometer (JENWAY 6715, Bibby Scientific Ltd UK). Negative control, without extract was used as the blank. The total flavonoid content was determined as milligram of rutin equivalent by using an equation that was obtained from standard rutin graph as:

$$Absorbance = 0.0144 \times total\ flavonoid\ (mg/g\ rutin) + 0.0556$$

Determination of Reducing Power of *Allium cepa* L. Extract

The reducing power of sprouted and boiled onions extract (sprouted for 0 day, 2 days, 4 days, 6 days, 8 days and 10 days), (0 min, 2 mins, 4 mins, 6 mins, and 8 mins) was determined according to the method of Oyaizu (1986). Briefly 1 ml of the extract was mixed with 2.5 ml of phosphate buffer (0.2M, pH 6.6) and potassium ferricyanide ($K_3Fe(CN)_6$) (2.5ml, 1 %). The mixture was incubated at 50°C for 20 mins. Then, trichloroacetic acid (10%), 2.5ml) was added to the mixture and centrifuged. Finally, the upper layer (2.5ml) was mixed with distilled water (2.5ml) and ferric chloride ($FeCl_3$) (0.5ml; 0.1%). The absorbance of the solution was measured at 700nm in spectrophotometer (JENWAY 6715, Bibby Scientific Ltd UK). Blank was prepared with all the reagents without extract. Higher absorbance of the reaction mixture indicated that the reducing power is increased. Ascorbic acid was used as standards.

Determination of 1,1-diphenyl-2-picrylhydrazil (DPPH) Radical Scavenging Activity by *Allium cepa* L. Extract

The free radical scavenging activity of sprouted and boiled onions extract was determined using DPPH. The method used is similar to the method previously discribed by Gadow *et al*., (1997) with slight modification. In details, 2ml of methanol solution of DPPH radical in concentration of 0.05 mg/ml and 1 ml of plant-extract were placed in cuvettes. The mixture was shaken vigorously and allowed to stand at room temperature for 30mins. Then the absorbance was measured at 517mm against methanol as blank in spectrophotometer (JENWAY 6715, Bibby Scientific Ltd UK).

The DPPH radical concentration was calculated suing the following equation:

$$DPPH\ Scavenging\ Effect\ (\%) = \frac{A_o - A_1}{A_o} \times 100$$

Where A_0 is the absorbance of the negative control (2ml of methanol solution of DPPH radical + ml of 5% (DMSO) and A_1 is the absorbance of reaction mixture or standards. Ascorbic acid was used as the standard.

Determination of 2,2-azino-bis 3-ethylbenzothiazoline-6-sulfonic Acid ($ABTS^+$) Radical Scavenging Activity by *Allium cepa L.* Extract

This was done by using the $ABTS^+$ free radical decolourization assay developed by Re *et al*. (1999) with some modification. Briefly, pre-formed radical monocation of ABTS was generated by reacting ABTS (7mm) with 2.45mm potassium per sulphate ($K_2S_2O_8$). The mixture was allowed to stand for 15 hours (overnight) in the dark at room temperature. The solution was diluted with ethanol to obtain the absorbance of 0.6 ± 0.2 units at 750mm. The plant extracts were separately dissolved in ethanol to yield a concentration of 1mg/ml. An aliquot of 20µl of ethanolic test solution of each sample was added to 180µl of ABTS free radical cation solution. The absorbance, monitored for 5mins was measured spectrophotometically at 750nm (JENWAY 6715, Bibby Scientific Ltd UK). All measurements were performed in triplicate. Ascorbic acid was used as the standard.

Determination of Ascorbic acid (vitamins C) on *Allium cepa* L. Extract

The titrimetric method reported by Plummer, (1978), was used for the determination of Vitamin C content. Briefly, 5ml of diluted extract was measured into a boiling tube 1ml of glacial acetic and was added. Then the mixture was titrated with 0.1mg/ml 2, 6-dichlorophenolindophenol solution. A 5ml solution of 0.022mg/ml vitamin c solution was used as standard. Titre values of samples were compared with this value to obtain their vitamin c equivalent.

STATISTICAL ANALYSIS

The results obtained were recorded as mean ± SEM (n=3). Significant difference was tested using Analysis of Variance (ANOVA) and Tukey Kramer Multiple Comparison Test. The Instat-GraphPad statistical software package was used for data analysis. The difference was considered statistically significant when $P<0.05$.

III. Results

Table 1: Reducing Power (mg/AAE) of Different Concentrations of Methanol Extract of Sprouted Onions

Data are presented as mean ± standard error mean (SEM) of triplicate determinations; mean values with different alphabetical superscript within the same row suggests a statistically significant difference ($P<0.05$).

Result on the reducing power of varying concentrations of methanol extract of onions sprouted for different days is presented in Table 4.7 above. The reducing potential was found to be concentration dependent up to 0.4 mg/ml. From the EC_{50} values, the strongest reducing power was exhibited by the extract from onion bulbs sprouted for 6 days while the weakest reducing power was noticed with day 8 (EC_{50} = 0.73) and day 10 (EC_{50} = 0.74).

Time (mins)	Methanol Extract Concentrations (mg/ml)					
	0.1	0.2	0.3	0.4	0.5	EC_{50}
0	$9.31^{a} \pm 0.06$	$19.30^{b} \pm 0.04$	$27.77^{c} \pm 0.06$	$35.04^{d} \pm 0.03$	$17.51^{e} \pm 0.05$	0.57
2	$18.16^{a} \pm 0.03$	$24.66^{b} \pm 0.00$	$36.86^{c} \pm 0.03$	$38.79^{d} \pm 0.00$	$24.44^{e} \pm 0.03$	0.53
4	$22.12^{a} \pm 0.05$	$31.60^{b} \pm 0.03$	$45.57^{c} \pm 0.02$	$48.88^{d} \pm 0.03$	$32.40^{e} \pm 0.00$	0.39
6	$29.43^{a} \pm 0.06$	$38.21^{b} \pm 0.02$	$51.17^{c} \pm 0.03$	$56.82^{d} \pm 0.01$	$32.92^{e} \pm 0.00$	0.31
8	$8.43^{a} \pm 0.02$	$21.83^{b} \pm 0.03$	$25.30^{c} \pm 0.03$	$27.67^{d} \pm 0.00$	$24.03^{e} \pm 0.03$	0.73
10	$7.65^{a} \pm 0.03$	$19.53^{b} \pm 0.03$	$23.46^{c} \pm 0.02$	$26.97^{d} \pm 0.03$	$19.72^{e} \pm 0.02$	0.74

Table 2: Reducing Power (mg/AAE) of Different Concentrations of Methanol Extract of Boiled Onions

Data are presented as mean ± standard error mean (SEM) of triplicate determinations; mean values with different alphabetical superscript within the same row suggests a statistically significant difference ($P<0.05$).

Result on the reducing power of varying concentrations of methanol extract of onions boiled for different time is presented in Table 4.2 above. As was observed in table 4.1, as concentration of the sample extracts increased from 0.1 to 0.4 mg/ml there was a concomitant increase in Reducing Power; beyond this concentration, a significant decline was recorded.The EC_{50} values show that boiling resulted in decreased reducing potential of methanol extracts of onion samples.

Time (mins)	Methanol Extract Concentrations (mg/ml)					
	0.1	0.2	0.3	0.4	0.5	EC_{50}
0	$9.31^{a} \pm 0.06$	$19.30^{b} \pm 0.04$	$27.77^{c} \pm 0.06$	$35.04^{d} \pm 0.03$	$17.51^{e} \pm 0.05$	0.57
2	$6.34^{a} \pm 0.03$	$10.50^{b} \pm 0.03$	$16.67^{c} \pm 0.28$	$21.25^{d} \pm 0.00$	$12.29^{b} \pm 0.28$	0.96
4	$5.50^{a} \pm 0.03$	$7.04^{b} \pm 0.01$	$11.67^{c} \pm 0.03$	$14.67^{d} \pm 0.02$	$8.30^{e} \pm 0.02$	1.50
6	$2.69^{a} \pm 0.04$	$4.34^{b} \pm 0.00$	$7.57^{c} \pm 0.04$	$11.77^{d} \pm 0.02$	$6.69^{a} \pm 0.03$	1.67
8	$1.88^{a} \pm 0.05$	$3.07^{a} \pm 0.03$	$5.78^{b} \pm 0.00$	$9.89^{c} \pm 0.08$	$4.89^{d} \pm 0.01$	1.93

Table 3: DPPH Radical Scavenging Activity (%) of Different Concentrations of Sprouted Onions Extract

Data are presented as mean ± standard error mean (SEM) of triplicate determinations; mean values with different alphabetical superscript within the same row suggests a statistically significant difference ($P<0.05$).

Effect of varying concentrations on the DPPH radical scavenging activity of methanol extract of sprouted onions is shown in Table 4.3 above. The DPPH scavenging activity was found to be concentration dependent up to 0.4 mg/ml. At 0.5 mg/ml, there was significant decline in the DPPH scavenging activity.Although there was gradual but significant increase in the DPPH radical scavenging activity as the days of germination increased up to the 8^{th} day, EC_{50} values show that methanol extract of samples germinated for four days was most effective in scavenging DPPH radical.

Days of Sprouting	Methanol Extract Concentrations (mg/ml)					
	0.1	0.2	0.3	0.4	0.5	EC_{50}
0	$27.16^{a} \pm 2.41$	$42.14^{b} \pm 1.03$	$62.62^{c} \pm 1.19$	$76.24^{d} \pm 0.60$	$66.38^{e} \pm 0.59$	0.24
2	$39.52^{a} \pm 8.60$	$63.10^{b} \pm 0.60$	$70.01^{c} \pm 0.01$	$82.14^{c} \pm 3.57$	$72.03^{d} \pm 1.20$	0.15
4	$60.18^{a} \pm 8.26$	$74.17^{a} \pm 1.19$	$86.31^{b} \pm 0.59$	$97.93^{c} \pm 0.60$	$81.53^{a} \pm 0.60$	0.01
6	$68.57^{a} \pm 4.12$	$82.38^{b} \pm 0.59$	$98.12^{a} \pm 0.59$	$109.00^{c} \pm 1.03$	$93.24^{d} \pm 0.60$	0.04
8	$94.52^{a} \pm 2.38$	$108.35^{b} \pm 0.58$	$118.76^{c} \pm 1.19$	$135.19^{a} \pm 30.40$	$103.36^{d} \pm 0.00$	0.23
10	$69.43^{a} \pm 4.12$	$95.24^{b} \pm 0.60$	$102.79^{c} \pm 0.00$	$116.33^{d} \pm 0.60$	$97.60^{e} \pm 0.60$	0.06

Table 4: DPPH Radical Scavenging Activity (%) of Different Concentrations of Boiled Onions Extract

Data are presented as mean ± standard error mean (SEM) of triplicate determinations; mean values with different alphabetical superscript within the same row suggests a statistically significant difference ($P<0.05$).

Effect of varying concentrations on the DPPH radical scavenging activity of methanol extract of boiled onions is presented in Table 4.4 above. As was observed in table 4.3, as concentration of the sample extracts

increased from 0.1 to 0.4 mg/ml there was a concomitant increase in the DPPH scavenging activity; beyond this concentration, a significant decline was recorded.The EC_{50} values show that boiling diminished the ability of methanol extracts from boiled onions to scavenge DPPH radical.

Time (mins)	Methanol Extract Concentrations (mg/ml)					
	0.1	0.2	0.3	0.4	0.5	EC_{50}
0	27.16^{a}±2.41	42.14^{b} ± 1.03	62.62^{c} ± 1.19	76.24^{d} ± 0.60	66.38^{e} ± 0.59	0.24
2	22.91^{a} ± 2.38	36.55^{b} ± 0.59	43.31^{c}± 32.74	56.19^{d} ± 0.60	37.62^{e} ± 0.60	0.35
4	19.48^{a} ± 2.38	27.07^{b} ± 1.03	35.24^{c} ± 0.60	41.55^{d} ± 0.59	29.60^{e} ± 0.60	0.51
6	14.38^{a} ±2.38	20.24^{b} ± 0.60	22.50^{c} ± 1.03	29.91^{d} ± 0.59	21.69^{e} ± 0.60	0.83
8	9.24^{a} ± 2.38	12.69^{b} ± 0.60	15.62^{c} ± 0.60	17.03^{d} ± 0.60	14.48^{e} ± 0.59	1.63

Table 5: $ABTS^{+}$ Radical Scavenging Activity (%) of Different Concentrations of Sprouted Onions Extract

Data are presented as mean ± standard error mean (SEM) of triplicate determinations; mean values with different alphabetical superscript within the same row suggests a statistically significant difference ($P<0.05$).

The effect of concentrations on the ABTS+ radical scavenging activity of the differently sprouted onions is presented in Table 4.5 above. The ABTS scavenging activity was found to be concentration dependent up to 0.4 mg/ml. At 0.5 mg/ml, there were significant decline in the ABTS scavenging activity. Just as in table 4.1, although highest ABTS radical scavenging activity was observed on the 8^{th} day of sprouting, EC_{50} values however, shows that sprouting for four days was most effective. The lowest antioxidant activity (EC_{50} = 0.16) was noticed with the unsprouted.

Days of Sprouting	Methanol Extract Concentrations (mg/ml)					
	0.1	0.2	0.3	0.4	0.5	EC_{50}
0	46.47^{a}±0.16	51.14^{b} ± 0.16	59.34^{c} ± 0.06	67.48^{d} ± 0.00	64.31^{f} ± 0.16	0.16
2	49.97^{a}±0.16	55.07^{a} ± 0.16	63.08^{b}± 0.16	71.50^{c} ± 0.56	65.06^{d} ± 0.06	0.11
4	56.90^{a}±0.06	62.75^{b} ± 0.06	69.56^{c} ± 0.12	76.84^{d} ± 0.12	71.34^{e} ± 0.01	0.002
6	59.90^{a}±0.06	67.60^{b} ± 0.16	75.24^{c} ± 0.12	89.69^{a} ± 0.00	82.69^{d} ± 0.06	0.02
8	70.63^{a}±0.11	76.87^{b} ± 0.06	83.66^{a} ± 0.11	97.87^{d} ± 0.00	86.17^{e} ± 0.06	0.11
10	61.39^{a}±0.06	66.96^{b} ± 0.06	73.44^{c} ± 0.16	85.79^{d} ± 0.16	79.48^{a} ± 0.06	0.02

Table 6: $ABTS^{+}$ Radical Scavenging Activity (%) of Differently Boiled Onions Extract

Data are presented as mean ± standard error mean (SEM) of triplicate determinations; mean values with different alphabetical superscript within the same row suggests a statistical significant difference ($P<0.05$).

The effect of concentrations on the ABTS+ radical scavenging activity of the differently boiled onions is presented in Table 4.6 above. As the concentration of the sample extracts increased from 0.1 to 0.4 mg/ml there was a concomitant increase in the ABTS scavenging activity; beyond this concentration, a significant decline was recorded ($P<0.05$).The EC_{50} value of ABTS radical scavenging activity of the boiled onions extract follows the same pattern as shown in table 4.2. Boiling resulted in effectiveness of the extract in scavenging ABTS radicals.

Time (mins)	Extract Concentrations (mg/ml)					
	0.1	0.2	0.3	0.4	0.5	EC_{50}
0	46.47^{a}±0.16	51.14^{b} ± 0.16	59.34^{c} ± 0.06	67.48^{d} ± 0.00	64.31^{f} ± 0.16	0.16
2	43.47^{a} ± 0.06	49.37^{b} ± 0.06	56.78^{c} ± 0.06	65.83^{d} ± 0.06	59.90^{e} ± 0.12	0.19
4	40.66^{a} ± 0.06	45.38^{b} ± 0.06	50.83^{c} ± 0.06	58.78^{d} ± 0.12	55.08^{e} ± 0.07	0.27
6	36.21^{a} ± 0.12	41.32^{b} ± 0.06	47.90^{c} ± 0.12	53.41^{d} ± 0.00	49.48^{e} ± 0.06	0.34
8	29.30^{a} ± 0.06	37.05^{b} ± 0.06	42.70^{c} ± 0.11	47.44^{d} ± 0.06	43.47^{e} ± 0.06	0.43

IV. Discussion

Allium crops are among the most widely consumed vegetables on a global basis and onion (*Allium cepa*) has long been used for medicinal purposes, owing to its anti-inflammatory and antimicrobial properties (Shitole and Wadaskar, 2014). Sprouting is a simple technological method that is used to germinate seeds and has been reported to improve the nutritive value of seeds (Amal *et al*., 2007). Boiling is an important food processing method which makes some nutrients such as carbohydrates and proteins more digestible (Rodrigues *et al*., 2009). Boiling also leads to the loss of some phytochemical components found in plants such as quercetin glycosides (a flavonoid) which leach into the boiling water (Ioku *et al.*, 2001).

The results for phytochemical (total phenols, flavonoids, ascorbic acid) analyses and in vitro antioxidant assays show that sprouting for up to 8 days resulted in a significant ($P<0.05$) increase in all the parameters tested. This was followed by a slight but significant decrease at the 10^{th} day of sprouting. Generally, it was observed that sprouting for about four days had the highest EC_{50}, which means that sprouting for four

days was most effective in terms of antioxidant activities. Boiling for up to 8 minutes resulted in significant losses in the phytochemical parameters tested. Similar trends were observed with the antioxidant activities such as reducing power, DPPH and ABTS radicals scavenging activities.

Studies by Shan *et al*. (2005), Wu *et al*. (2006), Wong *et al*., (2006), and Sharma (2014) have earlier reported that phenolic compounds in spices and herbs significantly contribute to their antioxidant properties. They also reported that plant polyphenols are multifunctional; they can act as reducing agents, hydrogen-donating antioxidants, and singlet oxygen quenchers. The results presented in the present study follow the reported pattern of Shitole and Wadaskar (2014). They sprouted for 0, 7 and 15 days and reported an increase in the total phenolic content of sprouted onions till day 7 and then further decrease with further sprouting for 15 days. In this present study sprouting was done for 0, 2, 4, 6, 8 and 10 days. This made it possible to determine when maximum antioxidant activities are achieved.

During sprouting, although phytochemical contents and antioxidant activities peaked at day 8, effective concentration (EC_{50}) was optimal at day 4. Recently, Majid *et al*. (2016) studied the effect of sprouting on the physicochemical, antioxidant and flavonoid profile of onion varieties. They observed a significant ($P< 0.05$) increase in total flavonoid content of sprouted onions compared to the raw ones. Therefore, sprouting of onions for between four to eight days may be a method to increase the total flavonoid content of onions to harness an improved antioxidant potential of the onions.

From Table 4.5, the reducing power of all sprouted onion extracts was increased proportionately with the day of sprouting with the highest being observed on Day 6. Further increase above this day resulted in a decrease in the reducing power of the onions.This trend follows the same pattern with ascorbic acid. Ascorbic acid may therefore play a role in the reducing potential of onion extracts. Lean *et al.*(1999) asserted that plant polyphenols can act as reducing agents. Thus, higher phenolic compound possessed higher reducing potential.

In the DPPH radical scavenging activity, there was gradual but significant increase as the days of germination increased up to the 8^{th} day, EC_{50} values show that methanol extract of samples germinated for four days was most effective in scavenging DPPH radical. These findings are in accordance withearlier published data about antioxidant activities of garlic extracts(Nuutila et al., 2003). The strongest antioxidant activity was noticed with the onion extract sprouted for 4 days. This agree with the study of Prakash *et al.* (2007), who earlier reported that increasing the concentration of Kalitur seed extract showed a high antioxidant activity against free radicals damage on DNA. Cowie *et al.*(2008)recorded a significant ($P<0.05$) increase in the free radical-scavenging activity of onion and ginger as concentration of the extract and days of sprouting increased which corroborates the result of this study. According to Smith and Adanlawo (2014), 2,2-diphenyl-1-picril-hydrazil (DPPH) radical is a stable free radical that shows a maximum absorption at 517nm and is widely used to evaluate the free radical scavenging ability of natural compounds. As the electron becomes paired off in the presence of a free radical scavenger, the absorption vanishes; the resulting decolourization is stoichiometric with respect to the number of electrons taken up (Ayoola *et al*., 2008). Thefindings from the present studies contradict the results of Juániz *et al.* (2016) where the DPPH antioxidant activity of onion increased with boiling time. Ioko *et al*., (2001) claimed that phytochemical component found in plants such as quercetin glycosides (a flavonoid) which is the major component of red onions outer layers leach into the boiling water. The studies of Guk *et al*. (2012) earlier reported that the DPPH radical scavenging activities of red pepper were reduced by 60.5%. In the present study, DPPH radical scavenging activities were reduced by 65% with methanol extract, 72% and 75% with aqueous and chloroform extract respectively.HPLC analyses of the specific phenolic composition has shown that onions, especially the outer layers of the red variety contain quercetin and quercetin has been known to act by scavenging free radicals, chelating transition metal ions, and inhibiting oxidases (De Groot and Rauen, 1998; Lean *et al.*, 1999).

ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) is a chemical compound used to observe the reaction kinetics of specific enzymes (Shin *et al*., 2000). A common use for it is in the enzyme-linked immunosorbent assay (ELISA) to detect for binding of molecules to each other. ABTS is also frequently used by the food industry and agricultural researchers to measure the antioxidant capacities of foods (Huang *et al*., 2005). This study follows the reported pattern of Muhammad *et al*. (2015) who earlier reported that the ABTS value of sesame sprouts powder increased with increasing sprouting days. ABTS value is an indicator of antioxidant activity and onions sprouts is shown to have good antioxidant potential (Pasko *et al.*, 2009). This study also agree with the study of Guk *et al*. (2012) who earlier reported that the ABTS radical scavenging activities of red pepper was reduced by 39.8~55.7%. In the present study, ABTS radical scavenging activities was reduced by 36% with methanol extract, 37% and 41% with aqueous and chloroform extract respectively. This falls within the same range reported earlier by Guk *et al*. (2012).The results in this study disagree with those reported by some authors where no losses or increase in the ABTS antioxidant activity of onion after boiling, frying and griddling were found (Jiménez- Monreal *et al.*, 2009; Pellegrini *et al*., 2009).

V. Conclusion And Recommendation

This study revealed that sprouting of onions may be a way of improving the antioxidant potential of onions. It is recommended that onions be sprouted for between 4 – 6 days before processing for consumption. Whether the improved antioxidant potential of bulbs resulting from sprouting could help Overcome losses resulting from cooking is suggested as a topic for further studies.

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References

[1]. **Amal, B.K., Aurang, Z., Nizakat, B., Shahid, A.K. and Mohammad, S.K. (2007).** Influence of Germination Techniques on Phytic Acid and Polyphenols Content of Chickpea (*Cicerarietinum* L.) sprouts. *Food Chemistry*.**104**: 1074–1079.

[2]. **Augusti, K.T (1996).** Therapeutic Values of Onion (*Allium cepa* L.) and Garlic (*Allium sativum* L.). *Indian Journal of Experimental Biology*.**34**: 634–640.

[3]. **Ayoola, G.A., Coker, H.A.B., Adesegun, S.A., Adepoju-Bello, A.A., Obawaye, K., Ezennia, E.C. and Atangbayila, T.O. (2008).** Phytochemical Screening and Antioxidant Activities of Some Medicinal Plants used for Malaria Therapy in South-western Nigeria. *Tropical Journal of Pharmaceutical Research*.**7**(3): 1019–1024.

[4]. **Cowie, H., Hutson, N., Oztug, O. and Myers, C. (2008).** The Impact of Peer Support Schemes on Pupils' Perceptions of Bullying, Aggression and Safety at School. *Emotional and Behavioral Difficulties*.**13**(1): 63–71.

[5]. **De Groot, H., &Rauen, U. (1998).**Tissue injury by reactive oxygen speciesand protective effects of flavonoids.*Fundamental and Clinical Pharmacology*.**12**: 249–255.

[6]. **Gadow, A., Hansmana, C.F. and Joubest, E. (1997).**Comparison of the Antioxidant Activity of Aspalathin with Date of other Plants, Phenols of Rooibos Tea (*Aspalathuslinearis*) and Alpha-tocopherol.*Journal of Agriculture and Food Chemistry*.**45**: 632–638.

[7]. **Guk, H., Young, J.S., Seongeung, L., Junsoo, L. and Seon, M.Y. (2012).**Effects of Different Cooking Methods on the Antioxidant Properties of Red Pepper.*Journal of Preventive Nutrition and Food Science*.**17**(4): 286–292.

[8]. **Huang, D., Ou, B. and Prior, R.L. (2005).** The Chemistry Behind Antioxidant Capacity Assays. *Journal of Agricultural and Food Chemistry*.**53**: 1841–1856.

[9]. **Ioku, K., Aoyama, Y., Tokuno, A., Terao, J., Nakatani, N. and Takei, Y. (2001).**Various Cooking Methods and the Flavonoid Content in Onion.*Journal of Nutritional Sciences and Vitamin*.**47**: 78–83.

[10]. **Ismail, A., Marjan, Z.M. and Foong, C.W. (2004).**Total Antioxidant Activity and Phenolic Content in Selected Vegetables.*Food Chemistry*.**87**: 581–586.

[11]. **Jiménez-Monreal, A. M., García-Diz, L., Martínez-Tomé, M., Mariscal, M., and Murcia, M. A. (2009).** Influence of cooking methods on antioxidant activity of vegetables. *Journal of Food Science.***74**(3): 97–103.

[12]. **Juaniz, I., Iziar A. L., Estibaliz, H., Gema, P., Jose M. M., Concepcion, C., Maria-Paz, D. P. (2016).**Influence of Heat Treatment on Antioxidant Capacity and (Poly) Phenolic Compounds of Selected Vegetables.*Journal of Food Chemistry*.**197**: 466-473.

[13]. **Kumud, K., Biju, C.M. and Augusti, K.T. (1990).**Antidiabetic and Hypolipidemic Effects of SMCS Isolated from *Allium cepa* Linn. *Indian Journal of Biochemistry and Biophysics*. **32**: 49–54.

[14]. **Larrauri, J.A., Rupérez, P. and Saura-Calixto, F. (1997).**Effect of Drying Temperature on the Stability of Polyphenols and Antioxidant Activity of Red Grape Pomace Peels. *Journal of Agricultural and Food Chemistry*. **45**: 1390–1393.

[15]. **Lean, M., Norrozi, M., Kelly, L., Burrns, J., Talwar, D., Satter, N. (1999).** Dietary Flavanoids protect diabetic human lymphocytesagainst oxidant damage to DNA. *Diabetes.***48**: 176–181.

[16]. **Madrau, M.A., Piscopo, A., Sanguinetti, A.M., Del Caro, A., Poiana, M., Romeo, F.V. and Piga, A. (2008).** Effect of Drying Temperature on Polyphenolic Content and Antioxidant Activity of Apricots. *European Food Research and Technology*. **228**(3): 441–448.

[17]. **Majid, S., Ajmer, S.D., Savita, S., Gulzar, A.N., and Vikas N. (2016).** Effect of Sprouting on Physicochemical, Antioxidant and Flavonoid Profile of Onions Varieties. *International Journal of Food Science and Technology*.**51**: 317–324.

[18]. **Meda, A., Lamien, C.E., Romilo, M., Millogo, J. and Nacoulma, O.G. (2005).** Determination of the Total Phenolic, Flavonia and Proline Contents in Burkina Faso Honeys as well as well all their Radical Scavenging Activity. *Journal of Food Chemistry*.**91**: 571–577.

[19]. **Muhammad, A.S., Fatima, I., Moazzam, R.K., Mian, A.M., Muhammad, S., Shahid, M. and Naila, S. (2015).** Effect of Sesame Sprouts Powder on the Quality and Oxidative Stability of Mayonnaise. *Journal of Food and Nutrition Research*. **3**(3): 138–145.

[20]. **Nicoli, M.C., Anese, M. and Parpinel, M. (1999).** Influence of Processing on the Antioxidant Properties of Fruits and Vegetables. *Trends in Food Science and Technology*. **10**: 94–100.

[21]. **Nuutila, A. M., Puupponen-Pimia, R., Aarni, M., and Oksman-Caldentey, K. M. (2003).**Comparision of antioxidant activities of onion and garlic extracts by inhibitionof lipid peroxidation and radical scavenging activity. *Food Chemistry*.**81**: 485–493.

[22]. **Ola-Mudathir, K.F and Maduagwu E.N. (2014).** Antioxidant Effects of Methanol Extract of *Allium cepa* Linn on Cyanide-induced Renal Toxicity in Male Wistar Rats. *Nigerian Journal of Physiological Sciences*. **29**: 147–151.

[23]. **Ola-Mudathir, K.F., Suru, S.M., Fafunso, M.A., Obioha, U.E. and Faremi, T.Y. (2008).** Protective Roles of Onion and Garlic Extracts on Cadmium Induced Changes in Sperm Characteristics and Testicular Oxidative Damage in Rats. *Food and Chemical Toxicology*. **46**: 3604–3611.

[24]. **Oyaizu, M. (1986).** Studies on Production of Browning Reaction Prepared from Glucose Amine. *Japanese Journal of Nutrition*.**44**: 307–315.

[25]. **Pasko, P.**, **Barton, H., Zagrodzki, P., Gorinstein, S., Folta, M., Zachwieja, Z. (2002).** Anthocyanins, Total Polyphenols and Antioxidant Activity in Amaranth and Quinoa Seed and Sprouts During their Growth. *Journal of Food Chemistry*. **50**: 5445–5449.

[26]. **Pellegrini, N., Miglio, C., Del Rio, D., Salvatore, S., Serafini, M., &Brighenti, F. (2009).** Effect of Domestic Cooking Methods on the Total Antioxidant Capacity of Vegetables. *International Journal of Food Sciences and Nutrition*.**60**(2): 12–22.

[27]. **Plummer, P.S. (1978).** Note on the Palaeo Environmental Significance of the Nuccaleena Formation (Upper Precambrian), Central Flinders Ranges, South Australia. *Journal of the Geological Society of Australia*. **25**(7): 395–402.

[28]. **Prakash, D., Singh, B. N., &Upadhyay, G. (2007).** Antioxidant and Free Radical Scavenging Activities of Phenols from Onion (*Allium cepa*). *Food Chemistry*.**102**: 1389–1393.

[29]. **Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C. (1999).** Antioxidant Activity Applying an Improved ABTS Radical Cation Decolourization Assay. *Free radical Biology and Medicine*. **26**: 1231–1237.

[30]. **Rodriques, A.S., Perez-Gregorio, M.R., Garcia-Falcon, M.S. and Simal-Gandara, J. (2009).** Effect of Curing and Cooking on Flavonols and Anthocyanins in Traditional Varieties of Onions Bulbs. *Food Research International*. **42**(9): 1331–1336.

[31]. **Roy, M.K., Takenaka, M., Isobe, S. and Tsushida, T. (2007).** Antioxidant Potential, Anti-proliferative Activities, and Phenolic Content in Water-soluble Fractions of Some Commonly Consumed Vegetables: Effects of Thermal Treatment. *Food Chemistry*. **103**: 106–114.

[32]. **Shan, B., Cai, Y.Z., Sun, M. and Corke, H. (2005).** Antioxidant Capacity of 26 Spice Extracts and Characterization of their Phenolic Constituents. *Journal of the Agricultural and Food Chemistry*. **53**: 7749–7759.

[33]. **Sharma, N. (2014).** Free Radicals, Antioxidants and Disease. *Biological Medicine*. **6**: 214–216.

[34]. **Shin, J.A., Kim, S.H., Kim, U.K., Chae, J.J., Choe, S.J., Namkoong, Y., Kim, H.S., Park, Y.B. and Lee, C.C. (2000).** Identification of Four Novel Mutations of the Low-density lipoprotein Receptor Gene in Korean Patients with Familial Hypercholesterolemia. *Clinical Genetics*. **57**(3): 225–229.

[35]. **Shitole, A.A. and Wadaskar, A.V. (2014).** Onion and Ginger Sprouting is associated with *Research*. **5**(4): 4586–4589.

[36]. **Slinkard, K. and Singleton, V.L. (1997).** Total Phenolic Analyses: Automatic and Comparison with Manual Method. America Journal EnolVitic. **28**: 49–55.

[37]. **Smith, Y.A. and Adanlawo, I.G. (2014).***In vitro* and *in vivo* Antioxidant Activity of Saponin Extracted from the Root of *Garcinia kola* (Bitter Kola) on Alloxan-Induced Diabetic Rats. *World Journal of Pharmacy and Pharmaceutical Sciences*. **3**(7): 8–26.

[38]. **Tomaino, A., Cimino, F., Zimbalatti, V., Venuti, V., Sulfaro, V. and De Pasquale, A. (2005).**Influence of Heating on Antioxidant Activity and Chemical Composition of some Spice Essential Oils. *Food Chemistry*. **89**: 549–554.

[39]. **Toor, R.K. and Savage, G.P. (2006).** Effect of Semi-drying on the Antioxidant Components of Tomatoes. *Food Chemistry*. **94**: 90–97.

[40]. **Vali, L., Stefanovits-Banyal, E., Szentmihalyi, K., Febel, H., Sardi, E., Lugasi, A., Kocsis, I. and Blazovics, A. (2007).** Liver-protecting Effects of Table Beet (*Beta vulgaris* var. Rubra) during Ischemia-reperfusion. *Nutrition*. **23**(2): 172–178.

[41]. **Wong, C., Li, H., Cheng, K. and Chen, F. (2006).** A Systematic Survey of Antioxidant Activity of 30 Chinese Medicinal Plants Using the Ferric Reducing Antioxidant Power Assay. *Food Chemistry*. **97**: 705–711.

[42]. **Wu, C.Q., Chen, F., Wang, X., Kim, H.J., He, G.Q., Haley-Zitlin, V. and Huang, G. (2006).** Antioxidant constituents in feverfew (*Tanacetumparthenium*) extract and their chromatographic quantification. *Food Chemistry*. **96**: 220–227.

[43]. **Yang, J., Guo, J. and Yuan, J. (2008).** *Invitro* Antioxidant Properties of Rutin. *Food Science and Technology*. **41**: 1060–1066.

Ebhomienlen, J. O, et. al. "The Effects of Different Concentrations of Sprouted And Boiled Onions Extracts On the Antioxidant Potentials Of Onions (Allium cepa L.)." *IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)*, 7(4), (2021): pp. 18-25.