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Effect of Blanching Time on Total Phenolic, Antioxidant Activities and Mineral Content of Selected Green Leafy Vegetables

Oluwaseun P. Bamidele^{1*}, Mofoluwaso B. Fasogbon², Olalekan J. Adebowale³ **and Adeyemi A. Adeyanju4**

1 Department of Food Science and Technology, Federal Polytechnic, P.M.B. 420, Offa, Nigeria. ² Department of Food Science and Technology, Obafemi Awolowo University, P.M.B. 13, Ile-Ife, Nigeria. ³

Department of Food Technology, Federal Polytechnic, P.M.B. 50, Ilaro, Nigeria. ⁴ Department of Food Science, University of Pretoria, Private Bag X20, Hatfield, Pretoria, South Africa.

Authors' contributions

This work was carried out in collaboration between all authors. Author OPB designed the study, performed the statistical analysis. Author MBF wrote the protocol and wrote the first draft of the manuscript. Author OJA managed the analyses of the study. Author AAA managed the literature searches. All authors read and approved the final manuscript.

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Original Research Article

ABSTRACT

Effect of blanching time in hot water (0, 5, 10 and 15 min) was carried out on six green leafy vegetables popularly consumed in Nigeria namely*; Cnidoscolus asconitifolius*, *Talinum triangular*, *Celosia argentea*, *Amaranthus hybridus*, *Vernomia anygdalina*, and *Telfaria occidentalis*. The dry matter, total phenolic content, antioxidant activity and mineral content of the green leafy vegetables were analyzed. Blanching at temperature of 90°C and different times decreased the dry matter content of the vegetables; 5 min blanching significantly ($p > 0.05$) increased the total phenolic (280.6 – 980.6 mgGAE/100 g db) and antioxidant activity (25.1 – 95.1 mg/100 g Trolox equivalent)

**Corresponding author: E-mail: bampet2001@yahoo.co.uk;*

in all the vegetables. However, further increase in the blanching time caused a significant reduction in total phenolic content, antioxidant activities and mineral content of all the vegetables. With these results, it could be concluded that blanching time for these vegetables should range between 1 to 5 min to prevent the loss of health benefiting compound present in them.

Keywords: Green vegetables; blanching; mineral; total phenolic; antioxidant activities.

1. INTRODUCTION

In West Africa, mostly Nigeria, has some massive indigenous vegetables which are consumed as food and use for medicinal purposes [1]. Most of these green leafy vegetables (GLVs) are reportedly cheap and affordable [2] and are a much-needed component of human diet as they offer a range of required nutrients and they constitute a significant component of diets in many Nigerian homes. Aside from adding variety to the menu, they also add flavour; taste, colour and aesthetic appeal to what would otherwise be a monotonous diet. From the nutritional standpoint, they serve as sources of various important nutrients (protein, minerals, and vitamins) and other phytochemicals with potential healthpromoting properties [3]. These nutritional qualities, as well as sensory attributes of green leafy vegetables, make it appeal to numerous consumers thereby enhancing its potential in contributing to food security and improve health. This makes vegetables be potentially useful in contributing to food security and improving human health.

In Nigeria, GLVs are not usually consumed in their fresh form unlike fruits; however, they usually undergo varieties of processing operations such as blanching, boiling, freezing, salting, sun-drying and juicing to improve their nutritional as well as sensory qualities [4]. These processing operations, if not well handled can also pose a serious threat to the nutritional qualities (vitamins, minerals) and healthpromoting the potential of vegetables, apart from improving their palatability, bioavailability of phytochemicals, and preservation as well as removing microbes and other toxins [3].

Blanching can be explained as the mild heat treatment given to fruits and vegetables before freezing, canning or drying so as to reduce the bulkiness, to aid easy packaging and inactivate enzymes. It helps in modifying texture while maintaining the nutritional value of food [5]. The blanching process also helps in eliminating air that could have been trapped in the cells of fruits

and vegetables [6], preserving the colour and flavour [7,8]. It is usually done traditionally using hot water or steam [9] to eliminate both the acid components and bitterness of the vegetables. There had been much information on the effect of blanching on the nutritional composition of vegetables; however, little information is available on the effect of blanching time on the nutritional and antioxidant properties of some of the Nigeria indigenous GLVs. Hence, this study aimed at determining the effect of various blanching time on nutritional and antioxidant activities of six indigenous green leafy vegetables in Nigeria.

2. MATERIALS AND METHODS

All the fresh GLVs were purchased from Owode market in Offa Kwara State, Nigeria. Identifications of the vegetable varieties were confirmed at the Herbarium, Department of Botany, Obafemi Awolowo University Ile-Ife, Nigeria.

2.1 Vegetable Sample Preparation

The vegetables were sorted by manually removing the inedible part with a sharp knife and washed under running tap water. About 1200 g of each vegetable (*Cnidoscolus asconitifolius, Talinum trangulare, Celosia argentea, Amaranthus hybridus, Vernomia anygdalina and Tefaria occidentalis*) was divided into four equal portions. One portion was retained raw (0 min) while others were blanched at three different times (5, 10, and 15 min).

About 100 g of previously washed vegetables was blanched in thermostatically regulated hot water bath (Gallenkamp, United Kingdom) already set at 90°C (Modified [10]). The blanching was done at 5, 10, and 15 mins. The samples were drained off and cooled rapidly under running tap and sun dried. The dried samples were homogenized using (Hamilton Beach, India) blender for 3 min and packed in air tight container, kept at -20 ℃ until further analyses.

2.2 Dry Matter Determination

The dry matter content was determined by weighing about 4 g of homogenized samples (as triplicate) into the crucibles, and dried in a convection oven (Labex, South Africa) at 70°C for at least 2 days until a constant weight was achieved [11]. The calculation was done on a dry basis.

2.3 Determination of Total Phenolic Content

The modified method of Velioglu et al. [12] was used to determine the total phenolic content (TPC) of the samples. 1 g of the homogenized samples was extracted with aqueous methanol 80% on a shaker for 2 h. 100 µl of the extract (1 mg/ml) was poured into a test tube and 0.75ml of Folin-Ciocalteu reagent (Diluted 10-fold with deionized water) were added and mixed. After resting the mixture for 5 min at ambient temperature (24°C), 0.75 ml of 6% (w/v) sodium carbonate was added and mixed gently. The absorbance was read at 725 nm using Hewlett Packard UV-VS spectrophotometer (HP, New Jersey, USA), after standing at room temperature for 90 min. The result was expressed as mgGAE/100 g.

2.4 Determination of Antioxidant Activity

DPPH (2, 2-diphenyl-2-picryl-hydrazyl) radical method was used to determine antioxidant activity of the extracted samples. The modified method of Zhang and Hamauzu [13] was adopted. Sample extracts (0.5 ml) was measured inside a test tube and 1.5 ml of 0.1 mM DPPH radical was added, vortexed and placed in the dark cupboard for 1 h. before reading the absorbance. The wavelength of 517 nm was used to measure the absorbance after calibrating the spectrophotometer (HP, New Jersey, USA) with absolute methanol. The result (antioxidant activity) was expressed as percentage inhibition of the radical (DPPH).

2.5 Mineral Content Determination

Minerals content (Sodium, Potassium, Magnesium, Calcium and Iron) of the vegetables were determined using AOAC [14] method 970.30. The mixture of concentrated nitric acid, sulphuric acid and perchloric acid (10:0:5:2, v/v) was used to digest the dried vegetable samples and atomic absorption spectrophotometer (GBC 904AA; Germany) was used to read the absorbance at wavelength of 880 nm. $KH_{2}PO_{4}$ (Merck, Mumbai, India) served as the standard.

2.6 Statistical Analysis

All experiments were repeated three times. Data were analyzed using IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp. One-way analysis of variance (ANOVA) was performed to determine significant differences due to blanching time. The means were separated using the least significant difference (LSD) test.

3. RESULTS AND DISCUSSION

The result of the effect of blanching time on the dry matter content of the six GLVs is presented in Table 1. The dry matter content of the vegetables ranged between 3.2 and 3.6 g at 0 min, 1.4 and 3.2 g at 5 min, 0.7 to 1.6 g and 10 min and 0.3 and 1.2 g at 15 min of blanching. The dry matter content was observed to be least at 15 min and highest at 0 min blanching time. *Cnidoscolus asconitifolius* and *Vernomia anygdalina* have the highest value (3.6 g) at 0 min and *Talinum trangulare* have the least value (0.3 g) at 15 min blanching time. It can be said from the results that there was a decrease in the dry matter with an increase in blanching time. The decrease may be due to loss of some components (nitrogen compounds and vitamins) during an increase in blanching time. Njoroge et al. (2015) reported a loss in crude protein content of indigenous leafy vegetables blanched and dried using various drying method. They attributed the loss to leaching out of water soluble vitamins and nitrogen compound during blanching. Similarly, Irondi et al. [15] reported decrease in phenolic compounds in blanched leafy vegetables, and the decrease was attributed to leaching out of water soluble phenolic compounds.

Table 2, shows the results of total phenolic content (TPC) of all the samples (blanched GLVs). The total phenolic content of the vegetables ranged from 140.3 to 840.3 mg GAE/100 g for 0 min, 280.3 to 980.6 mg GAE/100 g for 5 min, 189.1 to 790.1 mg GAE/100 g for 10 min and 89.4 to 620.4 mg GAE/100 g for 15 min. *Talinum trangulare* have the least value for all the treated samples (89.4 to 140.3 mg GAE/100 g) and *Vernomia anygdalina* have the highest value (520.4 to

980.6 mg GAE/100 g) with significant difference $(p > 0.05)$ among the values. The values reported in this study are higher than those reported by Oboh, [16] and Achinewhu, [17] for some tropical green leafy vegetables in Nigeria which were within the range of 21.3-98.8 mg GAE/100 g. These higher values recorded for some of the blanched GLVs are an indication that vegetables are rich source of phenolic compounds. Blanching for 5 min, however, causes significant increase ($p < 0.05$) in the total phenolic content of the blanched vegetables with *Vernomia anygdalina* having the highest increase (16.7%).

Increase in the total phenolic content of the vegetables may be attributed to the reduction of enzyme-mediated polyphenol degradation (complete inactivation of native polyphenol oxidase). The increase in total phenolic content could also be due to the release of bound phenolic acids from the breakdown of cellular constituents of the plant cell walls in the leafy vegetable [18]. This initial increase in the total phenolic content of the vegetable correlate with the report of Liu et al. [19] where they reported that cooling or blanching could increase phenol contents in vegetables.

However, a further increase in the time of blanching $(10 - 15$ mins) of the GLVs caused a significant reduction in the total phenolic content of the vegetables. This reduction might be due to the degradation of phenolic compounds by heat or their leaching out from the vegetable tissues into the blanching water. Francisco et al. (2010) reported that some phenolic compounds are known to be insoluble form in combination with plant cell wall components. During blanching (high temperature 90°C and time (10-15 min), disruption of the cell wall of the plant may occur leading to leaching out of the soluble phenolic

compound. Irondi et al. (2016) reported a decrease in the phenolic content of blanched *Adansonia digitata.* They attributed the decrease in phenolic content to oxidation and leaching out of phenolic compound during blanching. It may also be possible that the phenolic compound forms a complex with other components present in the vegetables such as protein, carbohydrate and some anti-nutritional compounds thereby reducing their extractability [20].

The results of antioxidant activities of all the samples are presented in Table 3. The result showed that antioxidant activities of the samples were directly proportional to the total phenolic content of the sample with a correlation value of R = 0.96 for *Cnidoscolus asconitifolius*, 0.93 for *Talinum trangulare*, 0.85 for *Celosia argentea*, 0.97 for *Amaranthus hybridus*, 0.88 for *Vernomia anygdalina* and 0.98 for *Teliferia occidentalis* (Fig. 1). The antioxidant activities (DPPH) of the samples ranged between 19.4 to 89.4 mg TE/100 g sample at 0 min, 25.1 to 95.1 mg/100 g Trolox eq. at 5 min, 19.0 to 68.0 mg TE/100 g sample at 10 min and 11.2 to 62.2 mg TE/100 g sample at 15 min. *Talinum trangulare* have the least radical scavenging power (11.2 to 25.1 mg/ TE/100 g sample) while *Vernomia anygdalina* have the highest radical scavenging power (62.2 to 95.4 mg TE/100 g sample). The scavenging power of the of the vegetable extracts reduced with the blanching time $(0 > 5 > 10 > 15$ min). Various researchers have reported the same trend of decrease in radical scavenging power of blanched leafy vegetables [21,22,15]. Most of the researchers attributed the decrease in the radical scavenging power to loss of water soluble and thermal degradation of phenolic compounds during blanching. Also, the report of Sikora et al. [23] showed that processing time and size of vegetable contribute to polyphenolic degradation.

Data are expressed as means ± SD of triplicate experiments (on dry basis) mean values in a row with different letters are significantly different at p ≤0.05

Blanching	Cnidoscolus	Talinum	Celosia	Amaranthus	Vernomia	Telfaria		
time (min)	asconitifolius	trangulare	argentea	hvbridus	anygdalina	occidentalis		
Ω	640.2° + 1.4	$140.3^{b} + 2.6$	340.3° ±2.3	$540.3^{\circ}+3.1$	840.3° ± 3.5	740.3° ±4.5		
-5	780.6° ±2.6	280.6° ±1.9	480.6° ±2.1 680.6 ^{\circ} ±3.6		980.6° ±3.4	780.6° ±5.2		
10	610.1 ^b \pm 2.9	189.1° + 1.6	390.1° ±2.6 490.1 ^b ±2.9		$790.1^b \pm 3.6$	690.1 $^{\circ}$ ±3.6		
15	$590.4^a \text{±} 3.6$	$89.4^a + 1.2$	$202.4^a \texttt{\textsterling}3.6$ 320.4 $^a \texttt{\textsterling}2.8$		520.4° ±4.2	620.4° ±2.8		
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Table 2. Effect of different blanching time on total phenolic content (mg/100 g gallic acid equivalent in dry basis) of some vegetable in Nigeria

Data are expressed as means ± SD of triplicate experiments (on dry basis) mean values in a row with different letters are significantly different at p ≤ 0.05

Table 3. Effect of different blanching time on antioxidant activity of (mg TE/100 g sample db) some vegetable in Nigeria

Blanching time (min)	Cnidoscolus asconitifolius	Talinum trangulare	Celosia argentea	Amaranthus hybridus	Vernomia anygdalina	Telfaria occidentalis
0	47.6° + 1.1	$19.4^\circ \pm 0.6$	39.4° ±0.2	49.4 $^{\circ}$ +0.2	89.4° ±0.8	52.4° ±0.4
-5	56.2° ±0.6	25.1° ±0.3	45.1° ±0.6	55.1° ±0.3	95.1° ±0.6	56.2° ±0.3
10	$41.6^b \pm 0.7$	19.0° ±0.4	$30.0^b \pm 0.4$	38.0^{b} ± 0.4	68.0^{b} ±0.5	$51.1^b \pm 0.5$
15	$37.8^a \pm 0.5$	$11.2^a \pm 0.2$	$21.2^a + 0.5$	22.2° +0.5	62.2° ±0.4	$47.2^a \pm 0.6$

Data are expressed as means ± SD of triplicate experiments (on dry basis) mean values in a row with different letters are significantly different at p ≤0.05

Fig. 1. Correlation graph between total phenolic content and antioxidant activities of blanched and raw green leafy vegetables

Increase in blanching time from 10 to 15 min, caused a significant ($p < 0.05$) reduction in the antioxidant activity of the vegetables. The reduction in antioxidant activity ranged between 2.48 and 23.85% with *Vernomia anygdalina* have the least and *Talinum trangulare* have the highest at both 10 and 15 min. This may be attributed to increase in blanching time which allowed more phenolic compound to leach out and thermal degradation of heat sensible

phenolic compound [23]. This result was similar to the results reported by Irondi et al. [15] on *Adansonia digitata* blanched at 80°C for 10 min.

Table 4 showed the effect of blanching time on the mineral content of the GLVs. The results indicated that the mineral content varied among of each vegetable samples. The level of sodium in *Cnidoscolus asconitifolius* was not detected, while *Talinum trangulare* and *Celosia argentea* were very low in sodium (0.3 and 5.2 mg/100 g) when compared with other samples. The potassium value was higher in *Telfaria occidentalis* (130.2 mg/100 g) and *Vernomia anygadaline* (73.3 mg/100 g). The remaining samples contain a smaller amount of potassium such as 11.1 mg/100 g for *Amarantus hybridus*, 6.1 mg/100 g for *Talinum trangulare*, 3.9 mg/100 g for *Celosia argentea* and 2.8 mg/100 g for *Cnidoscolus asconitifolius.* The magnesium

content of *Telifera occidentalis* was the highest (160.7 mg/100 g) while *Celosia argentea* has the lowest (1.4 mg/100 g). *Vernomia anygdalina* have the highest value for calcium (71.5 mg/100 g) and *Talinum trangulare* have the least (2.4 mg/100 g). *Amaranthus hybridus* have the highest content of Iron (87.5 mg/100 g), while *Talinum trangulare* and *Celosia argentea* have the least (0.4 mg/100 g each).

Data are expressed as means ± SD of triplicate experiments (on dry basis) mean values in a row with different letters are significantly different at p ≤0.05.

ND is not detected

The results clearly indicated that blanching caused a significant reduction (P value) in the levels of all the minerals determined. In fact, blanching of *Talinum trangulare* for just five minutes caused a significant reduction (P value) in the levels of sodium, magnesium and iron to an undetectable level. This was similar for magnesium and iron content *Celosia argentea.* The result also showed that loss in minerals increased as the blanching time increased, although the effect varied among the samples. The reduction in the mineral content of all the GLV after blanching for various times could be attributed to leaching out of most water-soluble minerals during blanching. Since most of these minerals are present in the cell wall of the plant; disruption of the plant cell wall will leach them out into the water during blanching. It can also be said that the longer the GLVs stay in the water during blanching the more the reduction in minerals. This result is in line with the findings of [24] and [25] who reported a decrease in the mineral content of *Vernomia amygadalia* and *Cnidoscolus aconitifolius* respectively.

4. CONCLUSION

Blanching time has a great effect on total phenolic content, antioxidant activities and minerals content of all the green leafy vegetables examined in this study. It can be concluded that the more the blanching time for these vegetables, the less the mineral content and the potential health benefit.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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