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Antioxidant Degradation in Six Indigenous Okra *Abelmoschus esculentus* (L) Moench Varieties During Storage in Nigeria

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Abstract: The aim of this study is to determine the antioxidant-phytoconstituents of 6 indigenous okra *Abelmoschus esculentus* (L) moench varieties in Nigeria and the rate of deterioration of these antioxidants during storage. Okra pods were stored in polypropylene bag for up to 10 days to maintain pod quality parameters. The vitamin C, total phenol, reducing power and iron chelating activity of the Okra were determined on alternate days. The vitamin C content of these okra varieties ranges from 43.42 mg/100 g 'Ikaro' okra to 50.01 mg/100 g 'Okene' okra, during storage there was a significant ($p>0.05$) reduction in the vitamin C content of all the indigenous okra varieties. The total phenolic ranges from 0.106% 'Auchi' okra to 0.095% 'Benin' okra. However, there was no significant ($p>0.05$) difference in the total phenolics of these indigenous okra varieties but in storage; there was a significant ($p>0.05$) difference in the rate of loss of the total phenol contents and reducing power, with 'Lokoja' okra having the lowest percentage loss. Iron chelating activity of all the okra varieties ranges from 86.54% for 'Ikaro' to 77.47% for 'Auchi' okra, during storage there was a significant ($p>0.05$) decrease in the iron chelating activity of all the indigenous okra at the end of the storage period with 'Benin' okra having the lowest percentage loss of activity (19.85%).

Key words: Vitamin C, total phenol, reducing power, iron chelating, Okra

INTRODUCTION

Since, many degenerative human diseases have been recognized as being a consequence of free radical damage, there have been many studies undertaken on how to delay or prevent the onset of these diseases (Oboh, 2005a). Antioxidants are powerful free radical scavengers in the body, while free radicals are highly reactive chemical substances such as peroxide, hydroxyl radical, singlet oxygen etc. that travel around in the body and cause damage to the body cells (Alia *et al*., 2003). Free radical damage is one of the most prominent causes of devastating diseases that are responsible for killing many people in the world, such as cardiovascular disease, which can manifest as heart attacks and cancer (Amic *et al*., 2003). Free radicals naturally occur in the body as a result of chemical reactions during normal cellular processes such as during the conversion of food into energy by the body (Oboh and Rocha, 2006a).

Antioxidant nutrients (found in foods) soak up all the excess energy that these free radicals have, turning them harmless particles or waste products that we can get rid of (Oboh, 2005a). Antioxidants are powerful substances that can neutralize free radicals before they damage the body cells (Oboh and Rocha, 2006a). Many phenolics, such as flavonoids have antioxidant capacities that are much stronger than those of vitamins C and E. Some evidence shows that flavonoids could protect membrane lipids from oxidation. A major source of flavonoid is vegetables and fruits (Amic *et al*., 2003).

Okra *Abelmoschus esculentus* (L) moench is a tall annual dicotyledon related to cotton and thought to be of African origin. It is still found growing wild along the river Nile Egypt and in Ethiopia (Kochhar, 1986). French colonist carried okra to the new world soon after 1700. Okra is a power house of valuable nutrients. Nearly half of which is soluble fibre in the form of gums and pectins and soluble fibre helps to lower serum cholesterol, reducing the risk of heart diseases. The other half is insoluble fiber, which helps to keep the intestinal tract healthy (Jeff, 2002).

In Nigeria, produce including okra are stored and transported in polypropylene bag, during transportation due to poor road network from the outlying villages to the market it could take 2-4 days for the produce (okra) to get to the markets and to the final consumers. There is a dearth of information on antioxidant-phytoconstituents present in okra.

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The aim of this study is to determine the antioxidant-phytoconstituents of 6 indigenous okra varieties in Nigeria and the rate of deterioration of these antioxidants during storage. This data will be used to estimate the loss in these antioxidants when stored in polypropylene bag during transportation.

MATERIAL AND METHOD

Okra plant *Abelmoschus esculentus* L moench used for this study were 'Benin', 'Auchi', 'Ikaro', 'Akure', 'Okene' and 'Lokoja' indigenous okra. Plant was grown on a fallow land of 5 years measured 14 ×14 m located in a farm in Ifon, Ondo state. The experiment was laid out in a Randomized Complete Block Design (RCBD). Each experimental unit was planted on side of ridges. Spacing was 0.9 between and 0.45 m within rows. Seeds were planted three per hole and thinned to one per stand 2 Weeks after Planting (WAP) given a plant population of about 24, 690 plants hG^{1}. Each potential okra pod was tagged on the day of anthesis (pollination) when the flower opened in order to determine the actual age of pods in days, they were harvested on the 8th day after anthesis using knife. Only pods free of apparent mechanical injuries, insect damage or disease were selected and put in polypropylene bag for storage at room temperature of (27±1°C). The okra pods were analyzed five times during storage period. All determinations were done in triplicate vitamin C, total phenol, reducing power and iron chelating activity.

The vitamin C content of the Okra were determined by A.O.A.C. (1990) method, described thus 5 g of the sample was extracted by 100 mL H_2O, 25 mL of 20% glacial acetic acid was added to 10 mL of the sample extract and titrated against standardized 2, 6 dichloroindophenol (0.05 g/100 mL) solution. The total phenol was determined by mixing 0.2 mL phenolic extract (0.2 g of the Okra extracted by 20 mL 70% Acetone) with 0.8 mL folin-ciocalteu reagent and 2 mL of 7.5% sodium carbonate. The mixture was diluted to 7 mL distilled water and the absorbance was measured after 2 h at 765 nm, the result was calculated as gallic acid equivalent (Pulido *et al*., 2000). The reducing power of the okra were determined by assessing the ability of the okra to reduce $FeCl_3$ solution as described by Iqbal *et al*. (2004), briefly 2.5 mL of the okra aliquot (0.5 g of the okra homogenized in 20 mL methanol) was mixed with 2.5 mL, 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1 g/100 mL potassium ferrocyanide, the mixture was incubated at 50°C for 20 min, thereafter, 2.5 mL, 10 mL/100 mL. Trichloroacetic acid was added and subsequently centrifuged at 650 rpm for 10 min, 5 mL of the supernatant was mixed with equal volume of water and 1 mL of 0.1 g/100 mL ferric chloride, the absorbance was later measured at 700 nm, a higher absorbance indicates a higher reducing power. Iron chelating activity was determined according to the method of (Kuda *et al*., 2004). The Okra Sample solution (0.1 mL) was pipetted into 10 mL test tube, 0.1 mL of distilled water and 0.025 mL of 2.5 mM iron, chloride were added, it was shaked thoroughly and the absorbance was taken at 550 nm (Abs1). 0.025 mL of 2.5 mM ferrozine was added to the mixture, it was allowed to stay for 20 min at room temperature; the absorbance was taken at 550 nm (Abs2).

Analysis of data: The results were statistically verified on the basis of analysis of variance, using (SAS, 2002). Mean separation were done where there is significant differences using Duncan multiple range test procedure as described in the SAS soft ware. Least Significant Difference (LSD) being calculated for the probability level of $p>0.05$.

RESULTS AND DISCUSSION

The result of the vitamin C content of these okra varieties and their rate of deterioration are shown in Table 1. Vitamin C contributes to the antioxidant properties of vegetables by protecting the membrane erythrocyte, maintaining the blood vessel flexibility and improving blood circulation in the arteries of smokers as well as flexibility the absorption of iron in the body (Oboh, 2005b). The vitamin C content of these okra varieties ranges from 43.42 mg/100 g 'Ikaro' okra to 50.01 mg/100 g 'Okene' okra, these results were within the same range with what Achinewhu (1983) and Oboh (2005a) reported for some tropical vegetables, this is an indication that indigenous okra are good sources of vitamin C. 'Okene' okra has a significantly ($p>0.05$) higher value of vitamin C. During storage there was a significant ($p>0.05$) reduction in the vitamin C content of all the indigenous okra varieties. The loss in vitamin C may be as a result of the activity of the enzyme ascorbate oxidase which strongly depends on the pH of the vegetable. The enzymes convert ascorbic acid to dehydroascorbic acid in stored produce. The highest level of reduction was recorded in 'Okene' okra with 73.12% loss in vitamin C content, while 'Ikaro' okra had the least loss in vitamin C content (67.31%). Albuquerque *et al*. (2005) and Evensen (1983) also reported decrease in ascorbic acid content of winter melon during storage period.

Plant phenolics are of particular importance during the postharvest period due to their role in both flavor and color (Kay, 1991). Phenolic phytochemicals inhibit

Table 1: Vitamin C degradation in indigenous okra stored in polypropylene bag in mg/100g

	Day	'Benin' okra	'Auchi' okra	'Ikaro' okra	'Akure' okra	'Okene' okra	'Lokoja' okra
	0	46.28±0.026c	48.92±0.038b	43.42±0.021d	48.73±0.029b	50.01±0.038a	49.87±0.029a
	2	18.36±0.035a	17.25±0.020b	17.36±0.053b	18.66±0.052a	17.26±0.036b	19.76±0.053a
Vitamin C	4	17.28±0.026b	16.16±0.010b	16.48±0.026b	17.31±0.010b	16.18±0.031b	18.56±0.051a
	6	16.48±0.026b	15.35±0.035b	15.88±0.017b	16.78±0.026b	15.38±0.036b	17.68±0.078a
	8	15.26±0.026b	14.14±0.000c	14.86±0.036b	15.29±0.026b	14.16±0.036c	16.62±0.026a
	10	14.39±0.035b	13.24±0.052c	14.19±0.010b	14.43±0.026b	13.44±0.044c	15.86±0.025a
	Loss (%)	68.90	72.93	67.31	70.38	73.12	68.19

Values with the same letter along the same row are not significantly different (p>0. 05)

Table 2: Degradation of total phenol in indigenous okra stored in polypropylene bag (%)

	Day	'Benin' okra	'Auchi' okra	'Ikaro' okra	'Akure' okra	'Okene' okra	'Lokoja' okra
	0	0.095±0.004a	0.106±0.007a	0.097±0.003a	0.098±0.003a	0.096±0.003a	0.103±0.002a
	2	0.082±0.003ab	0.071±0.003b	0.072±0.003b	0.085±0.003ab	0.071±0.003b	0.096±0.002a
Total	4	0.076±0.003b	0.064±0.003c	0.068±0.003bc	0.079±0.003b	0.065±0.003bc	0.089±0.003a
Phenol	6	0.068±0.003bc	0.055±0.003c	0.062±0.003bc	0.070±0.003b	0.058±0.003c	0.080±0.003a
	8	0.062±0.003b	0.048±0.003d	0.058±0.003bc	0.065±0.003b	0.051±0.003c	0.073±0.003a
	10	0.059±0.003b	0.044±0.003c	0.057±0.003b	0.062±0.003ab	0.047±0.003c	0.067±0.003a
	Loss (%)	37.89	58.49	41.23	36.72	51.04	34.95

Values with the same letter along the same row are not significantly different (p>0. 05)

Table 3: Reducing power degradation in indigenous okra stored in polypropylene bag at 700 nm

	Day	'Benin' okra	'Auchi' okra	'Ikaro' okra	'Akure' okra	'Okene' okra	'Lokoja' okra
	0	1.20±0.020a	1.16±0.035a	0.95±0.044a	1.16±0.052a	1.07±0.026a	1.02±0.026a
	2	0.74±0.026b	0.63±0.021b	0.64±0.026b	0.77±0.026b	0.63±0.026b	0.88±0.026a
Reducing	4	0.68±0.026b	0.56±0.084c	0.60±0.026c	0.71±0.026b	0.57±0.026c	0.81±0.026a
Power	6	0.56±0.026ab	0.43±0.026b	0.56±0.026ab	0.58±0.026ab	0.45±0.026b	0.68±0.026a
	8	0.48±0.026bb	0.34±0.026b	0.44±0.026b	0.51±0.026a	0.37±0.026b	0.59±0.026a
	10	0.46±0.026b	0.31±0.026c	0.44±0.026bc	0.49±0.021b	0.30±0.026c	0.55±0.026a
	Loss (%)	61.66	73.27	53.68	57.75	71.96	46.07

Values with the same letter along the same row are not significantly different (p>0. 05)

autoxidation of unsaturated lipids thus preventing the formation of oxidized low-density lipoprotein (LDL) which is considered to induce cardiovascular disease (Amic *et al*., 2003) The total phenol content of these indigenous okra varieties is presented in Table 2. The total phenolic ranges from 0.106% 'Auchi' okra to 0.095% 'Benin' okra. These values were lower to what Oboh (2005a) reported for *Amaranthus cruentus* and *telfairia occidentalis* (0.3%), they were however, within the range of value reported for ripe *capsicum pubesceus* (0.117%) 17 but higher than the values reported by Yang *et al*. (2002) for some varieties of commercial mushrooms (0.01-0.02%). However, there was no significant (p>0.05) difference in the total phenolics of these indigenous okra varieties. When this indigenous okra goes into storage, there was a significant (p>0.05) difference in the rate of loss of the total phenol contents, with 'Lokoja' okra having the lowest percentage loss of 34.95%. The reduction in the total phenolic content may be attributed to the fact that phenols are susceptible to oxidation by phenolases which converts them to quinones. These compounds are often extremely reactive and therefore, short lived. They quickly polymerized and/or react with hydroxyl groups of carbohydrates or thiol and other groups of proteins (Kays, 1991; Mason and Peterson, 1965).

It shows from Table 3 that 'Benin' okra had the highest capacity to cause the reduction of Fe to Fe with the reducing power of 1.20_{700nm} while 'Ikaro' okra has the least, though there was no significant (p>0.05) difference in the reducing power of all the indigenous okra varieties. The values reported in this research, compared well with the report of (Yang *et al*., 2002.) for variety of mushrooms and Oboh (2005a) for some tropical green leafy vegetables. During the storage of these indigenous okra varieties, it shows that there was a significant (p>0.05) decrease in the reducing power with 'Lokoja' okra having the lowest percentage loss of 46.07%. This reduction could be as a result of the reduction in the phenolic content of the okra in storage because most of the antioxidant activities in vegetables depend on the total phenolic content of the vegetable.

The result of the iron chelating activity of all the indigenous okra varieties analysed are shown in Table 4, the result indicated that iron chelating activity of all the okra varieties ranges from 86.54 for 'Ikaro' to 77.47% for 'Auchi' okra, there was no significant (p>0.05) difference in the iron chelating activity of 'Akure', 'Okene' and 'Lokoja' okra. The values reported in this research, compared well with the report of Oboh and Rocha (2006b) for *capsicum pubescens*. However, during storage there was a significant (p>0.05) decrease in the iron chelating activity of all the indigenous okra at the end of the storage period. At the end of the storage period 'Benin' okra had the lowest percentage loss of activity (19.85%). The loss in the iron chelating activity could be as result of the loss in the vitamin C and total phenolics of these okra varieties.

Table 4: Iron chelating activity in indigenous okra stored in polypropylene bag (%)

	Day	'Benin' okra	'Auchi' okra	'Ikaro' okra	'Akure' okra	'Okene' okra	'Lokoja' okra
Iron	0	78.24±0.026bc	77.47±0.026c	86.54±0.052a	80.19±0.010b	79.82±0.044b	81.07±0.026b
Chelat	2	72.34±0.026a	71.21±0.026a	71.34±0.026a	72.64±0.026a	71.24±0.026a	73.74±0.026a
Ing	4	68.28±0.026a	67.16±0.026a	67.48±0.026a	68.31±0.032a	67.18±0.026a	69.58±0.026a
Activty	6	66.30±0.026ab	65.17±0.026b	65.70±0.026b	66.60±0.026ab	65.20±0.055b	67.50±0.026a
	8	64.56±0.026a	63.42±0.026b	64.16±0.026ab	64.86±0.026a	63.46±0.026b	65.66±0.026a
	10	62.71±0.026a	61.56±0.032b	62.51±0.026a	63.01±0.017a	61.04±0.026b	62.17±0.026a
	Loss (%)	19.85	20.53	27.76	21.42	23.52	23.31

Values with the same letter along the same row are not significantly different (p>0. 05)

CONCLUSION

It is therefore, concluded that most of these village roads should be repaired so as to facilitate quick transportation of these okra varieties to the consumer in order for them to have access to much of these antioxidants.

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