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VARIATION IN THE ANTIOXIDANT ACTIVITY OF OKRA UNDER CULINARY PREPARATIONS

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ABSTRACT

Okra, a green vegetable of South India used in various food preparations. The present findings proved that the raw okra is rich in antioxidants, phenolics and reducing power compared to fried and boiled once. There was quantitative loss of antioxidants in both fried and boiled okra which is equal to that of fungal infected okra through the *in vitro* inoculation of *Fusarium verticilloides*, a common pathogen in okra seeds. Three fold differences were noticed in raw and boiled samples with respect polyphenols. DDPH assay also indicated the three fold differences among fresh and boiled samples, confirmed the high scavenging activity of raw than boiled once. The results proved there was loss of free radical scavenging activity, quantitatively, phenolics and reducing power due to boiling, which is equivalent to *Fusarium* infection in fruits. Hence, it is suggestible to prefer raw / half cooked fruits to save its essential nutrients.

KEYWORDS: Okra, Culinary Preparations, Antioxidant Activity

INTRODUCTION

Okra is a common vegetable crop in South India, being cultivated for its valuable nutritive values. The fruits are rich in mucilage, vitamins, dietary fibers, minerals, protein with high antioxidant activity. Natural antioxidants from dietary sources include phenolics and polyphenolic compounds, chelators, vitamins, enzymes, carotenoids and carnosine. Okra is routinely being consumed raw, fried, cooked, pickled manner. Such a plant is suffering from various diseases in the field especially due to seed-borne fungal pathogens. As the result, nutritive quality of okra getting depleted in various growth stages. Ultimately the quantitative yield is being depleted with respect to the antioxidant activity. On the other hand the culinary preparations like frying; cooking also causes the loss of antioxidants at varied levels. In view of this, in the present study the aqueous and organic (methanol) extracts of okra fruits at raw, fried, boiled conditions have been analyzed for their effects on antioxidant activity.

MATERIALS AND METHODS

Seed sample of Okra (*Abelmoschus esculentus* (L.) Moench.) was collected from V. C. farm(Agricultural Research Station), Mandya District of Karnataka State in India, subjected for through washing in tap water to remove surface borne dust and other fungal propagules and air dried under shade. Further, the seeds were plated equidistantly on three layers of wet blotters in the 9cm diameter Perspex plates and incubated for a period of one week at 22+/-2C under alternate cycles of 12/12 h light and darkness as per ISTA rules. On 8th day, the dominant fungus, *Fusarium verticillioides* that colonized on the seeds was isolated and its cultures were maintained in the Petri plants for further use. On the other hand, apparently healthy seeds were sown in the experiment plots in the rows; the plants raised were supplied with suitable

water and fertilizers. The fruits produced were harvested and subjected to culinary purposes. Accordingly, 1kg of fruits were harvested and cut into pieces and divided into four parts of 250g each. Each of 250g fruits were separately chapped subjected to boiling (with 50ml water), frying (with 20ml oil), where as one set was maintained raw. Fruits at early stages of development were sprayed with the spore suspension of *F. verticilloides* at the concentration of 500 spores/ml. After one week of inoculation the fruits were harvested and used for the extraction with water as well as methanol, separately.

25g of each fruit sample such as raw, boiled, fried and fungal inoculated were separately kept for drying in a hot air oven at 60C for overnight. Further, the dried samples were powdered using an electric blender. Then 25g powder of each sample was separately subjected for aqueous and methanol extraction. In each case 250ml of the solvent was added and subjected for rigorous shaking on a mechanical shaker at 1000rpm for 20 minutes. Further, each extract was filtered through Whatman No. 1 filter paper; the filtrates thus collected were separately kept in hot air oven at 60C for complete drying. The dried residue (powder) remained was separately collected in to a series of vials for further use. 10mg each of these powders was taken into the 1.5ml eppendroff tubes separately, to which 10ml of methanol was added and vortexed thoroughly and kept for further use for the analysis of antioxidant activity, polyphenol and reducing power.

Following the procedures of Naskar *et al.* (2011), the antioxidant activity of different samples was performed *in vitro* in triplicates for both aqueous and methanol extracts. In each case 10ml of the sample was pipette out into test tubes which were added with 3ml of methanol followed by the addition of 1ml of DDPH solution. These tubes were further incubated for 30 minutes under darkness. Then the O.D. was taken using a spectrophotometer at 517nm. The activity was expressed as % DDPH scavenging activity based on the following formula;

DDPH activity (%) = Absorbance of Control–Absorbance of sample/Absorbance of Control x 100

Where, extracts from raw samples were considered as corresponding controls.

In the other set, each sample was subjected to polyphenol extraction considering the procedures of Prabhakaran (2002). Accordingly, 10ml of each extract was added with 3.0ml of distilled water, this was followed by the addition of 200ul of Folic-Ciocalteau reagent. The solutions were mixed well for 5 minutes then added with 300ul of Na2CO3 solution and were incubated for 30 minutes in a hot air oven at 40C. After incubation the O. D. was taken at 765nm using a spectrophotometer. Finally, polyphenol concentration was determined comparatively.

Similarly, reducing power in each sample was determined based on the procedures of Yen and Chen (1995). For this purpose 10ml of each extract was added with 2.5ml of Sodium phosphate buffer (pH 6) along with 2.5ml of Potassium ferricyanide (1%). Such samples were incubated for 30 minutes at 50C in the hot air oven. To such incubated samples 2.5ml of Trichloro acetic acid (100g/l) was added separately, centrifuged at 1600 rpm for 10 minutes. The 2.5ml supernatant was added further with 2.5ml distilled water and 0.5ml of Ferric chloride (0.1%) solution. The O.D. was taken at 700nm spectrophotometrically. The data in all the cases were compiled and tabulated.

RESULTS AND DISCUSSIONS

The data represented in table 1 revealed the variable antioxidant activity with respect to sample types and extraction (Table 1). In all the samples antioxidant activity were recorded at different levels. However, raw samples showed high antioxidant activity, comparatively. The same recorded at three fold low in case of boiled samples, which was almost equal to that of fungal inoculated samples. These results indicated the loss of antioxidant activity due to prolonged

heating as well as sucumbance to fungal metabolites, which might have result in the formation of complexes. Due to boiling the tissue get softened, facilitated the release of antioxidants; as the result, under the continuous heating they will be broken down or get denatured.

Similarly, the phenolics and reducing power also found to reduce in the boiled as well as in the fungal inoculated samples (Table 2 and 3). During heating, the fibrous plant tissue gets softened by the dissolution of hemicelluloses, pectin and lignin. At the same time all other active inter and intracellular components get easily exposed to the surrounding and get diminished. This might be the possible reason for the reduced concentration of phenolics and reducing power in the fried, boiled and fungal inoculated samples. In contrast to this due to lack of heat energy the quantities of antioxidant, phenolics and reducing power remain not obstructed, comparatively.

The present findings are in support of the statement of Miguel (2009) who observed raw food rich in phenol, increase the antioxidant pool in the plasma. The antioxidant activity in the food items is correspondingly posse's rich phenolics due to their high redox potentials (Saeedeh and Urooj, 2006), these reports are in support of the present findings. So, information out of these studies indicated the antioxidants in row fruits, play a major role in physiology to prevent free radical generation and oxidation stress. The present findings suggest the use of okra fruits raw or mild cooked serves better source of antioxidants for the potential scavenging of free oxygen radicals exists during metabolic process. In support of this, similar findings were also reported by Jiménez-Monreal *et al*. (2009) with respect to nutrient loss in various vegetables based on the different methods of cooking.

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APPENDICES

Table 1: Variation in the Antioxidant Activity* In Culinary Preparation of Okra

Okra Fruit Samples	% Antioxidant Activity in Different Extract	
	Aqueous Extract	Methanol Extract
Raw	68	53
Fried	24	37
Boiled	14	40
Fungus inoculated	12	36

*Data based on the mean of triplicates.

Table 2: Effect of Culinary Preparation of Okra on Polyphenolic Content

Okra Fruit Samples	% Polyphenolic Content in Different Extract of Okra	
	Aqueous Extract	Methanol Extract
Raw	43	37
Fried	19	26
Boiled	12	22
Fungus inoculated	10	20

*Data based on the average of triplicate values.

Table 3: Relationship of Culinary Preparation of Okra with Respect to Reducing Power

Okra Fruit Samples	% Reducing Power in Different Extract of Okra	
	Aqueous Extract	Methanol Extract
Raw	0.58	0.78
Fried	0.18	0.50
Boiled	0.16	0.28
Fungus inoculated	0.12	0.20

*Values are based on the mean of triplicates.