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LEVELS OF TOTAL PHENOLIC AND FLAVONOIDS IN *Abelmoschus esculentus* L. FROM SOME IRRIGATION AREAS OF KANO STATE-NIGERIA

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ABSTRACT

Vegetables are rich in polyphenols, flavonoids, glucosinolates, vitamin C and their hydrolysis products which may have antioxidant and anticancer properties. This study was carried out to assess the level of total phenolic and flavonoids in selected vegetables from different sampling areas of Kano state-Nigeria. Total phenolic and flavonoid contents in the extracts were determined by Rhodanine and AlCl₃ reagents and their amount calculated as gallic acid equivalent (GAE) and rutin equivalent (RE) fresh weight respectively, using UV-VIS spectrophotometer. The result show that, the levels of total Phenolics and Flavonoid contents in okra (*Abelmoschus esculentus* L.) vegetable samples among seven different sampling areas (viz: Bebeji, Kura, Tiga, Minjibir, Tudun Wada, Garun Malam and Chalawa) of Kano-state, Nigeria were determined. Levels of Total phenolic content ranged from 33.33 ± 0.02 to 22.07 ± 0.02 mg of gallic acid equivalent per gram of fresh matter and for flavanoid ranged from 29.97 ± 0.03 to 10.97 ± 0.01 mg of rutin equivalent per gram of fresh matter. The highest content of total phenolic in Okra was found in Bebeji sample and highest content of flavonoid was found in Minjibir sample. The results obtained revealed that, the Okra samples analyzed are potential sources of the bioactive compounds analyzed.

Key words: Flavonoid, phenolic, okra, Irrigation areas.

INTRODUCTION

Vegetables have been used as a source of food and medicine due to their abundant chemical properties since time immemorial (Sinha *et al.*, 2010). Crude extracts of vegetables and other plant materials rich in phenol content and flavonoids are of boosting interest in food industry and medical field. Phenolic compounds have an aromatic ring that contains various attached substituent group such as hydroxyl (-OH), carboxyl (-COOH) and methoxy (-OCH₃) group and often other non-aromatic ring structures. Phenolics hinder oxidative degradation of lipids and thereby enhance the excellence and nutritional value of food. Phenolics possess a wide spectrum of biochemical activities such as antioxidant, anti mutagenic, anti carcinogenic as well as ability of modifying gene expression (Srivastava *et al.*, 2013). More than 400 flavonoids have been identified in different higher and lower plant species (Srivastava *et al.*, 2013). Flavonoids are a group of poly phenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action. Due to its antioxidant properties it can interfere with the oxidative process by reacting with free radicals chelating, catalytic metals and also by acting as oxygen scavengers (Srivastava *et al.*, 2013). OKRA (*Abelmoschus esculentus* L.) is an annual herb belonging to the family of *Malvaceae*, is one of the most important vegetables grown in Nigeria. It is

widely grown for its tender fruits and young leaves. It is easy to cultivate and grows well in both tropical and temperate zones, that is, it is widely planted from Africa to Asia, and from Southern Europe to America. Since the discovery of its nutrition, it is widely cultivated in north and south China, in recent years. It has been the preferred vegetable for the Olympic athletes of the Beijing Olympic Games (Haibing *et al.*, 2012). For its functional characters, it has some interesting names, such as 'green pana' in Japan and 'plant Viagra' in the USA (Kang *et al.*, 2010). Okra is becoming a lot more popular all over the world. Nutritionally, it has been reported that there are many useful substances in the seeds of okra, such as, flavones, polysaccharide, pectin, trace elements, and amino acids (Adelakun *et al.*, 2009). Modern medical research provides that the extract of the fruit has the ability of resisting fatigue, and has anti-aging and anti-oxidant properties (Arapitsas, 2008). With the larger consumer demand for functional food, much more attention is paid to okra by our society, for its special functional and nutrition value. There exist certain important medicinal attribute for okra, which is given to people suffering from renal colic, leucorrhoea, spermatorrhoea, chronic dysentery and general weakness. Due to high iodine content, the fruits are useful for controlling goiter and leaves of okra are used in Turkey for preparation of medicines to reduce inflammation (Singh *et al.*, 2007).

Except for a few report on the presence of the total or individual phenolics, there is a lack of information on the nature of phenolic acids in commercial varieties and plant parts of okra. They also contribute to the astringent taste and thus to overall flavor of the food. Besides, there exist reports showing pronounced physiological and biological properties of a number of phenolic acids, namely, tannic, gallic, caffeic and cinnamic acids. It is reported that vegetables constitute an important source of dietary intake of polyphenols and phenolic acids account for almost one third of the total intake of phenolics compound (Scalbert and Williamson, 2000).

MATERIALS AND METHODS

Plant Materials

Vegetable samples Okra (*Abelmoschus esculentus* L.), used in this study were obtained from Tudun Wada, Tiga, Bebeji, Garun Malam, Minjibir and Chalawa irrigation areas of Kano state, Nigeria.

Samples Pretreatment

The samples were properly washed with distilled water and air dried at room temperature. They were ground into fine powder and kept for analysis.

Preparation of Standard

A stock solution of Gallic acid was prepared by dissolving 10mg of gallic acid in 2 ml methanol and marking up to 10 ml volume, from this solution 1 ml aliquot was pipette out and diluted to 10 ml using methanol to get final concentration of 100 µg/ml. Working standards of 0, 5, 10, 15, 20, 25 and 30µg/ml. were prepared by serial dilutions.

A stock solution of rutin was prepared by dissolving 10mg of rutin in 2 ml methanol and marking up the volume to 10 ml, from this solution 1 ml was pipette out and diluted to 10 ml by using methanol to get final concentration of 100µg/ml. Working standards of 0, 5, 10, 15, 20, 25 and 30µg/ml were prepared by serial dilutions (Suman *et al.*, 2014).

Extraction of Total Phenolic Content

100g of each of the vegetable samples was finely ground. 10 ml of aqueous acetone (70%) was added to 400mg of the powder and suspended in water bath for 20 min at room temperature. The suspension was transferred to centrifuge for 10 min at 3000 rpm. The supernatant was diluted with methanol to achieve a volume of 10 ml and kept in an ice (supernatant A). Each plant sample was extracted three times.

Quantitative Determination of Total Phenolic Content

200µL of supernatant A was transferred to a test tube (4tube/sample) and dried. 600µL of sulfuric acid (0.2N) was added to the test tubes. To three tubes, 900µL of Rhodanine solution (0.667%) was added and to the fourth tube 900 µL of methanol was added. The fourth tube was set as a blank. After 10 min 600µL of potassium hydroxide solution (0.5N) was added to all of the test tube. 6 min later 13 mL of distilled water was also added. After 25 min the absorbance of red -purple solution was measured at 520 nm against the blank using Varian UV-Vis spectrophotometer (Cary 50 Bio UV-Vis Spectrophotometer, Varian).The concentration of total phenolic content in the test samples was obtained from the calibration plot of standard by interpolation

and expressed as mg gallic acid equivalent (GAE)/g of dried plant material. All the determinations were carried out in triplicate (Mahdi *et al.*,2011).

Extraction of Total Flavonoid

1g each of the dried vegetable samples was used for the preparation of the extract. Samples were extracted with 7.5 mL (95% v/v) ethanol at 40°C for 10 min; the extraction process was repeated thrice. The solvent was evaporated at 40°C. The dried extract was used for further analysis (Suman *et al.*, 2014).

Sample Preparation

50 mg of the extract was dissolved in 5 mL methanol and sonicate for 45 minutes at 40°C followed by centrifugation at 1000 rpm for 10 min. The clear supernatant solution was collected and used for analysis.

Determination of total flavonoid

0.6 mL of supernatant solution was separately mixed with 0.6 mL of 2% aluminum chloride. After mixing, the solution was incubated for 60 min at room temperature. The absorbance of the reaction mixtures was measured against blank at 420 nm wavelength with a Varian UV-Vis spectrophotometer model (Cary 50 Bio UV-Vis Spectrophotometer, Varian). The concentration of total flavonoid content in the test samples was obtained from the calibration plot by interpolation and expressed as mg rutin equivalent (RE)/g of dried plant material. All the determinations were carried out in triplicate (Suman *et al.*, 2014).

Results and Discussion

Figure 1 showed that, Okra from Bebeji had the highest amount of total phenolic content of 33.33 ± 0.03 mg/g GAE among the various samples evaluated, followed by Tudun wada sample 32.03 ± 0.05 mg/g GAE. Kura sample was found to have the least phenolic content of 22.07 ± 0.02 mg/g GAE compared to other samples analyzed. Various values have been previously reported for total phenolic contents of vegetables, which includes, 49.72 ± 2.24 mg/g GAE of total phenolic content in Okra (Rohman *et al.*,2010). Ebrahimzadeh *et al.*, (2009), reported the total phenolic content of 68.81 ± 3.90 mg gallic acid equivalent/g of extract in Okra. Jin-Yuan and Chinyun., (2007), reported the levels of total phenolic content in Mulberry and Onions, 1515.9 ± 5.70 mg gallic acid equivalents (GAE)/100g fresh matter of total phenolic in Mulberry and 310.8 ± 4.9 mg GAE/100 g fresh matter of total phenolic content in Onions. Hung *et al.*,(2012) also reported the total phenolic content in Carrot, Taro, Tomato, Beet root and Egg plant as 36.60 ± 0.50 , 93.50 ± 0.90 , 61.20 ± 0.70 , 229.20 ± 1.60 , 454.50 ± 0.50 µg GAE/ g sample respectively. In Figure 2 Minjibir sample had the highest amount of flavonoid content of 29.979 ± 0.036 mg/g RE in okra among the various sampling areas investigated. This is followed by Bebeji sample 25.313 ± 0.051 RE whereas Chalawa sample has the least flavonoid content of 10.979 ± 0.095 mg/g RE. Flavonoid content have been previously reported from vegetables, which include, 31.21 ± 8.64 mg/g RE of flavonoid content in okra (Rohman *et al.*, 2010).

Ebrahimzadeh *et. al.*, (2009), reported flavonoid content of 49.3 ± 2.2 mg/g quercetin equivalent in okra. Jin-Yuarn and Chin-yin., (2007), reported the levels of flavonoid content in mulberry and Onions, 250.10 ± 6.30 mg quercetin equivalents (QE)/100g fresh matter of total flavonoid content in mulberry,

310.8 ± 4.9 mg GAE/100mg quercetin equivalents (QE)/100 g fresh matter flavonoid content in onions. Hung *et. al.*,(2012) had earlier reported the total flavonoid contents of carrot, taro, tomato, beet root and egg plant as 11.2, 7.1, 14.7, 15.3, 42.1 μ g RE/g respectively.

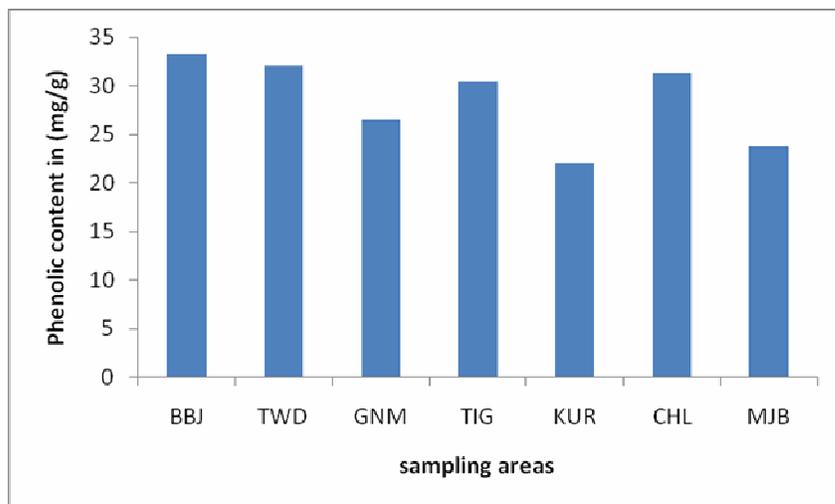


Figure 1: Mean concentration of total phenolic content in okra from the sampling areas
Key: BBJ=Bebeji TWD=Tudun Wada GNM=GarunMalam TIG=Tiga KUR=Kura CHL=Chalawa MJB=Minjibir

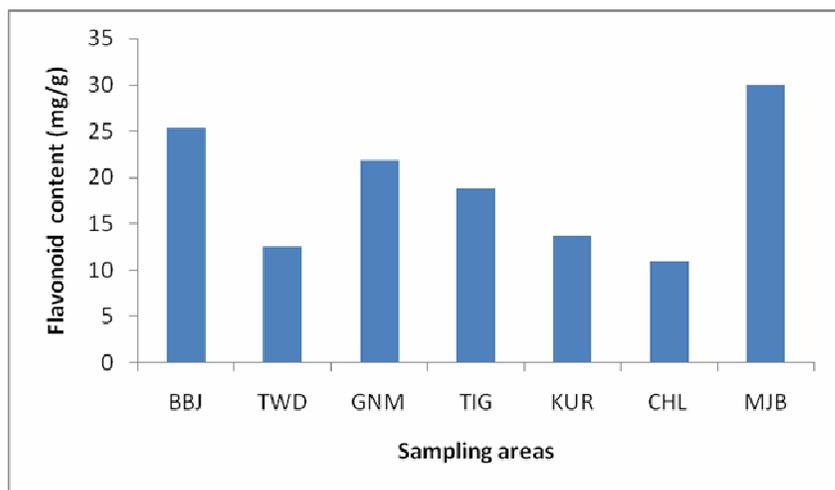


Figure 2: Mean concentration of flavonoid content in Okra from the sampling areas
Key: BBJ=Bebeji TWD=Tudun Wada GNM=GarunMalam TIG=Tiga KUR=Kura CHL=Chalawa MJB=Minjibir

Conclusion

The present study estimated the levels of total phenolic (TPC) and Flavonoid (TFC) contents in Okra vegetables widely available in Kano state, Nigeria. The Okra vegetable analyzed displayed high level of total phenolic (TPC) and Flavonoid contents (TFC). Therefore, this vegetable may be considered as good source of the bioactive compounds and antioxidant.

Recommendations

Further studies on the effective of antioxidants activity contained in this vegetable, other bioactive compound

and the mechanisms by which they protect against disease development are highly recommended.

Contribution

We were able to quantify the values of total phenolic and flavanoid in okra samples consumed in Kano State, Nigeria. The values will serve as a guide to farmers and consumers of the okra in deciding the types of crops that can be planted and possibly consumed.

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