



Nutritional composition and antioxidant capacity of twelve varieties of fresh immature Okra fruits (*Abelmoschus esculentus* L.)

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ABSTRACT

The aim of this study was to evaluate the nutrients content and antioxidant capacity of fresh immature fruits of twelve varieties from Okra (*Abelmoschus esculentus* L.). Protein and utilizable carbohydrate contents were evaluated by the method of Gornall (1949) and Dubois (1956), respectively. Atomic absorption spectrophotometry was used to determine the quantities of minerals present in the samples. The antioxidant activity was measured using FRAP test. The highest protein content is observed in the variety V₃ (36.055±0.017g/100g). That in utilizable carbohydrate is observed in the variety V₁₉ (9.2418 ± 0.014 g/ 100g). The zinc, iron and copper contents are relatively low. Varieties V₁₉ (60mg/kg), V₅₂ (40mg/kg) and V₄₂ (14mg/kg) showed the high contents of zinc, iron and copper, respectively. Evaluation of antioxidant activity in vitro of the extracts, from the different varieties of okra, by the reduction's method of the ferric ion (FRAP), showed interesting activities. The highest ferric ion reduction capacity was observed with the V₄₂ variety (62.93mmolEAA/g). All the results obtained revealed that immature fruits of okra's varieties V₅, V₁₆, V₅₂ and V₄₂ presented the higher nutritional and antioxidant potentials among all the varieties and could thus be recommended to children, women and older people for their diet.

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Introduction

Today, malnutrition is a major problem in most countries of sub-Saharan Africa, especially among mothers and children. Nearly one in three persons globally suffers from at least one form of malnutrition – undernutrition, nutrient deficiency, overweight or obesity – and a large part of the world's population is affected by diet related non-communicable diseases (FAO, 2017). The impacts of malnutrition on development, society, health and well-being are serious and lasting, for individuals and their families, for communities and for countries. Eliminating malnutrition in all its forms is imperative to break the intergenerational cycle of poverty, and reach the Sustainable Development Goals by 2030 (FAO, 2017).

Okra is a multipurpose crop due to its various uses of the fresh immature fruits, fresh leaves, buds, flowers, stems, and seeds. Nutritionally, the fresh immature fruits of okra plays an important role in the human diet because it contains carbohydrates, protein, fibers, minerals and vitamins, including vitamin C [Jarret et al., 2011; Sabitha et al., 2012].

It is beneficial to the digestive system and contributes to healthy intestinal functioning due to its high polysaccharide and microelement content [Adelakun et al., 2012, Ndjouenkeu et al., 1997]. Additionally, okra has a cholesterol-lowering effect [Rao et al., 1991] and may

therefore be a drug in the treatment not only of diabetes but also of excessive cholesterol. Therefore, promoting the consumption of fresh immature fruits of Okra could provide cheap sources of nutrients that can improve the nutritional status and reducing the prevalence of malnutrition especially among resource-constrained households and can also used as a means of dietary diversification. However, okra has been considered as a minor crop and there is no single information or published studies available about nutritional of fresh immature fruits of Okra grown in Benin.

Therefore, the aim of this study was to evaluate the nutrients content and antioxidant capacity of fresh immature fruits of twelve varieties from Okra (*Abelmoschus esculentus* L.) grown in Abomey-Calavi, Benin.

Methods

Plants Materials

Plants Materials: the fresh immature fruits of twelve varieties of Okra (*Abelmoschus esculentus*) were obtained from Department of Botanic, University of Abomey-Calavi, Republic of Benin, washed properly with distilled water and dried under shade at room temperature. This fresh immature fruits were blended into powdered form and stored in sterile containers until analysis.

Total protein content

The total protein content was determined by the Biuret assay (Gornall et al., 1949) using Beef albumin serum (BSA) as the standard. It is one commonly used method for determining the total protein in a sample is the Biuret method. The Biuret method is based on the complexation of Cu^{2+} to functional groups in the protein's peptide bonds. The formation of a Cu^{2+} -protein complex requires two peptide bonds and produces a violet-colored chelate product which is measured by absorption spectroscopy at 540 nm. 1 g of the sample was dissolved in 100 mL of distilled water. The mixture is then centrifuged at 3000 rpm for 15 min. 2 ml of the Gornall reagent were added to 0.5 ml of the filtrate obtained after maceration. 0.5 mL of distilled water was again added to this mixture and the whole is homogenized and incubated for 30 min. The absorbance of the mixture was then read at 540 nm against the white (Gornall reagent + distilled water) using a spectrophotometer. The amount of protein contained in the immature fruits of each variety was determined by a calibration curve from a concentration range of a Beef Serum Albumin solution (BSA).

Total sugars contents

Initially, each sample in the form of powder was slurried with distilled water using a solid to liquid ratio of 10% (0.1 mg/ml) and stirred at room temperature. Subsequently, the samples were centrifuged for 15 min 4000 rpm. The supernatant fluid was filtered through Whatman filter paper and the filtrate was used for determining total sugar by the respective procedure. Quantitative determinations of total soluble sugars were performed using a phenol-sulfuric acid colorimetric procedure (Dubois et al, 1956) based on the absorbance at 490 nm of a aromatic complex formed between phenol and the carbohydrate. The amount of Sugar present determined by comparison with a calibration using a reference standard of glucose on a spectrophotometer.

Determination of mineral contents

Minerals content analysis was determined according to AOAC, (2000). Copper (Cu), iron (Fe), and zinc (Zn) concentrations were measured by atomic absorption spectrophotometer.

Ferric-Reducing antioxidant power (FRAP) assay

Initially, 10 g of fresh immature fruit powder of each variety of *Abelmoschus esculentus* were extracted for 24 hours by maceration with 100 mL of ethanol-water (50:50) at room temperature under magnetic stirring. The extracts were filtered and the filtrate was concentrated by rotary vacuum evaporation at 40 °C until obtaining a solid residue. Reducing ability was performed using the procedure method described by Amoussa et al., 2015. Briefly, 2 ml of extracts (100 µg/ml)

were mixed with 2 ml of phosphate buffer (0.2 M, pH 6.6) and 2 ml of potassium ferricyanide (10 mg/ml). The mixture was incubated at 50°C for 20 min followed by addition of 2 ml of trichloroacetic acid (100 mg/l). The mixture was centrifuged at 3000 rpm for 10 min to collect the upper layer of the solution. A volume of 2 ml from each of the mixture earlier mentioned was mixed with 2 ml of distilled water and 0.4 ml of 0.1% (w/v) fresh ferric chloride. After 10 min reaction, the absorbance was read at 700 nm. Ascorbic acid was used to produce the calibration curve ($y = 0.028x - 0.024$; $R^2 = 0.995$). The iron (III) reducing activity determination was performed in triplicate and expressed in µMol Ascorbic Acid Equivalent (AAE)/g of extract.

Statistical analysis

Data were presented as mean \pm SD. The difference was considered statistically significant when the $p < 0.05$. All statistical analysis were conducted using Stata software version 12.0.

Results and Discussion

Total protein and total sugars contents.

The main functions of proteins are growth and replacement of lost tissues in the human body (Gemede et al., 2015). Nwofia et al. (2012) reported that diet is nutritionally satisfactory, if it contains high caloric value and a sufficient amount of protein. Table 1 shows the crude protein contents of the twelve varieties of Okra fresh immature fruits used in the study. The protein content of the fresh immature fruits of these okra varieties was varied significantly ($p < 0.05$) from $14,516 \pm 0,003$ g/100g in V_{23} to $36,055 \pm 0,017$ g/100 g in V_3 on dry weight basis. The mean value of the varieties obtained in the study is higher than the values reported by Adetuyi et al., 2011 (13.61–16.27 g/ 100g), Gemede et al., 2015 (10.25 - 26.16 g/100 g) and Nwachukwu et al., 2014 (4.81g/ 100 g). Ogungbenle and Omosola (2015) had also reported that the crude protein content of Okra fresh immature fruits is (23.4 g/100 g) which is higher than the fresh immature fruits of the varieties V_{19} , V_{23} , V_{32} , V_{41} , V_{42} and V_{52} in the present study. Okra can be considered as a high protein vegetable when compared with *Moringa oliefera* (4.2 g/100 g), Amaranthus (6.1 g/100 g), *Gnetum Africanum* (1.5 g/100 g), and Pterocarpus (2.0 g/100 g) (Nzikou et al. 2006). Effiong et al. 2009 and Ali, 2010 have shown that any plant foods that provides about 12% of their calorific value from protein are considered good source of protein. The fresh immature fruits of all varieties of Okra of present study, meet this requirements and this implies that these Okra fresh immature fruits can serve as a good source of protein. The utilizable carbohydrate contents of the fresh immature fruits of these okra varieties were varied significantly ($p < 0.05$) from one

Table 1. Total protein and utilizable carbohydrate content of the varieties from okra (dry weight basis).

Varieties	Total Protein (g / 100 g)	Utilizable carbohydrate(g / 100 g)
V_3	36.055 ± 0.017^a	7.3112 ± 0.002^a
V_5	35.732 ± 0.340^b	5.3426 ± 0.028^b
V_6	25.395 ± 0.0170^c	6.3563 ± 0.007^c
V_{16}	25.071 ± 0.034^d	7.1582 ± 0.001^d
V_{19}	17.356 ± 0.051^e	9.2418 ± 0.014^e
V_{23}	14.516 ± 0.003^f	8.9986 ± 0.014^f
V_{32}	16.641 ± 0.0170^g	6.0693 ± 0.0141^g
V_{33}	25.250 ± 0.008^h	2.8799 ± 0.042^h
V_{37}	26.825 ± 0.0510^i	3.4454 ± 0.155^i
V_{41}	14.649 ± 0.034^j	4.5707 ± 0.014^j
V_{42}	17.765 ± 0.017^k	6.0976 ± 0.014^k
V_{52}	22.193 ± 0.017^l	6.8949 ± 0.014^l

Means not followed by the same superscript letters in the same column are significantly different ($p < 0.05$). Data are expressed as mean \pm SE of replicate determinations (n = 2). V stands for variety.

variety to other. Table 1 shows the utilizable carbohydrate contents of the twelve varieties of fresh immature fruits of okra used in the study.

The utilizable carbohydrate content of fresh immature fruits of these okra varieties varied from 2.8799 ± 0.042 g/100 g to 9.2418 ± 0.014 g/100 g in varieties V₃₃ and V₁₉, respectively. The utilizable carbohydrate content of variety V₁₉ had higher (9.2418 ± 0.014 g/100 g), whereas varieties V₃₃ had the lowest (2.8799 ± 0.042 g/100 g). The mean value of the varieties obtained in the study is lower than the values reported by Gemedé et al., 2015 (36.66 to 50.97 g/100 g).

Table 2. Mineral concentrations of twelve varieties of Okra (dry weight basis).

Variétés	Zn (mg/ kg)	Fe (mg/ kg)	Cu (mg/ kg)
V ₃	ND	ND	ND
V ₅	47	30	10
V ₆	33	22	10
V ₁₆	56	33	13
V ₁₉	60	33	11
V ₂₃	53	32	13
V ₃₂	49	23	10
V ₃₃	54	31	12
V ₃₇	34	22	9
V ₄₁	ND	ND	ND
V ₄₂	49	32	14
V ₅₂	40	40	12

Table 3. Antioxydant activity obtained using the FRAP method.

Variétés	FRAP (Mmol EAA g ⁻¹)
V ₃	15.61
V ₅	19.44
V ₆	7.85
V ₁₈	28.57
V ₂₀	11.68
V ₂₃	19.73
V ₃₂	21.00
V ₃₃	30.75
V ₃₇	15.37
V ₄₁	21.48
V ₄₂	62.93
V ₅₀	45.04
AA	10.74

Mineral composition

Minerals are inorganic elements, some of which are essential nutrients. The major minerals (Ca, K, Na and Mg) and essential trace elements (Fe, Cu, Zn and Mn) play very important roles in human metabolism [Gorinstein et al., 2001]. Deficiencies of these minerals can lead to metabolic disorders and organ damage, leading to acute and chronic disease and ultimately death [Ozden et al., 2010]. The mineral composition of twelve varieties of okra is shown in Table 2.

Zinc is distributed widely in plant and animal tissues and occurs in all living cells. It functions as a cofactor and is a constituent of many enzymes like lactate dehydrogenase, alcohol dehydrogenase, glutamic dehydrogenase, alkaline phosphatase, carbonic anhydrase, carboxypeptidase, superoxide dismutase, retinene reductase, DNA and RNA polymerase (Soetan et al., 2010). Zn dependent enzymes are involved in macronutrient metabolism and cell replication (Hays and Swenson, 1985; Arinola, 2008). It is required for normal testicular development (Merck, 1986) and for functions of the taste buds. It is needed for tissue repair and wound healing, plays a vital role in protein synthesis and digestion, and is necessary for optimum insulin action as zinc is an integral constituent of insulin. It is an important constituent of plasma (Malhotra, 1998; Murray et al., 2000).

Zinc content in the fresh immature fruits of twelve varieties from okra is shown in Table 2. The content of Zinc varied between 33 mg/Kg in V₆ and 60 mg/ kg in V₁₉.

Zinc content of fresh immature fruits from V₁₉ had higher (60 mg/ Kg) while variety V₆ had the lowest (33 mg/ Kg) on dry weight basis. The values obtained in this study are higher than the values reported by Adetuyi et al., 2011 (12.9 mg/kg – 13.7 mg/ kg). However these values are similar than the values reported by Gemedé et al., 2015 (38.3 mg/ kg – 63.1 mg/kg).

Iron functions as haemoglobin in the transport of oxygen. In cellular respiration, it's functions as essential component of enzymes involved in biological oxidation such as cytochromes c, c1, a1 (Malhotra, 1998). Iron is an essential trace element for haemoglobin format on, normal functioning of central nervous system and in the oxidation of carbohydrates, protein, and fats (Kermanshah et al. 2014; Mlitan et al., 2014). Table 2 shows Iron content of the twelve varieties of Okra used in the study. The contents of Iron varied from 22 mg/kg in V₆ and V₃₇ to 40 mg/ kg in V₅₂. The Iron content of Okra variety V₅₂ had higher (40 mg/ kg) whereas the varieties V₆ and V₃₇ had the lowest (18.30 mg/100 g) on dry weight basis. The values obtained in this study were far higher than the value reported by Adetuya et al. (2011) which is varied from 8.7 mg/kg to 9.6 mg/ kg. However these values were far lower than the values reported by Gemedé et al., 2015 (183.0 mg/ kg to 366.8 mg/ kg). This indicates that fresh immature fruits of okra are a rich source of Iron.

Copper content of the varieties of Okra fresh immature fruit is shown in Table 2. In this study, the copper content is varied from 9 mg/ kg (V₃₇) to 14 mg/ kg (V₄₂). The copper content of variety V₄₂ had higher content (14 mg/ kg) while the variety V₃₇ had the lowest (9 mg/ kg) on dry weight basis. The values of this study is higher than the values reported by dos Santos et al., (2013) which is varied from 0.7 mg/kg to 2.14 mg/ kg.

Reducing power of fresh immature fruits extracts from *A. esculentus*

Several methods were used to determine the antioxidant activity of plants. Thus, our study involved one method to assess the antioxidant activity of fresh immature fruits of varieties from *A. esculentus*, namely, ferric reducing/antioxidant power (FRAP) analysis. In this assay, the compounds with reduction potential react with potassium ferricyanide (Fe³⁺) to form potassium ferrocyanides (Fe²⁺), which then react with ferric chloride to form ferric ferrous complex that is greenish in colour (Jothy et al., 2012). In the current study, Ferric Reducing Antioxidant Power (FRAP) of the extracts varied from 7.85 to 162.93 Mmol AAE g⁻¹ (table 3). These results reveal also the power reducing capacity of the varieties V₃ (15.61 mmol EAA.g⁻¹), V₅ (19.44 mmol EAA g⁻¹), V₁₆ (28.57 mmol EAA g⁻¹), V₁₉ (11.68 mmol EAA g⁻¹), V₂₃ (19.73 mmol EAA g⁻¹), V₃₂ (21 mmol EAA g⁻¹), V₃₃ mmol EAA g⁻¹), V₄₂ (62.93 mmol EAA g⁻¹) and V₅₂ (45.04 mmol EAA g⁻¹) are respectively better than the power reducing capacity of ascorbic acid (10.74 mmol EAA g⁻¹), used as standard. However, the variety V₆ (7.85 mmol EAA g⁻¹) have lower reducing power than ascorbic acid.

Conclusion

The study revealed that the fresh immature fruit of these varieties of Okra were found to be a good source of vital nutrients like crude protein, sugars, zinc, copper and iron. Specifically, the fresh immature fruits of varieties V₅, V₁₆, V₅₂ and V₄₂ contained significantly higher amounts of crude

protein, sugars, iron, copper and zinc than all other varieties and can be recommended as solution to alleviate malnutrition in the country.

Interestingly, all varieties except the variety V₆ showed the power reducing capacity better than the power reducing capacity of ascorbic acid.

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