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Effect of Boiling on the Anti-Oxidant Potential of Cocoyam [Colocasia Esculenta (Schot)] Inflorescence

Kalu emmanuel okechukwu

Department of crop science, Gregory University, Uturu

ABSTRACT

Effect of boiling on the anti-oxide potentials of Cocoyam inflorescence was determined. The sample was boiled for 10minutes and sundried for about five days. Unprocessed (fresh) samples served as the control. Samples were analysed for minerals and vitamins using standard analytical methods. Boiling had significant ($p<0.05$) reduction on antioxidant properties of cocoyam inflorescence. The mineral contents of the fresh sample were 60.70, 0.003 and 0.04 (mg/100g) for Zn, Fe and Cu respectively. Boiled sample showed 6.54, 1.12 and 0.68 (mg/100g) for Zn, Fe and Cu. Pro-vitamin A content of fresh sample was 348.91µg/dl while Vitamin E, B2 and C were 16.82, 12.59 and 27.21 mg/100g, respectively. Boiled samples recorded 11.28, 0.43, 0.59 and 0.09 for pro – vitamin A, vitamin E, B2 and C respectively. The antioxidant properties of cocoyam inflorescence showed 0.663, 0.529 and 0.308 reducing power respectively for fresh, vitamin C (control) and boiled samples. This study had demonstrated that boiling for 10 minutes and sun drying of cocoyam inflorescence is capable of improving the antioxidant properties of cocoyam inflorescence.

INTRODUCTION

The nutritive value of vegetables is based mainly on their richness in minerals and vitamin contents (Chima and Igyor, 2007). Green leafy vegetables also contain bioactive compounds (phytochemical) that have potentials in helping to reduce the risk of several deadly diseases in man (Okechukwu et al., 2019). Epidiemologic studies have shown that consumption of vegetables can protect humans against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species (Ames et al., 1983and Williams et al., 1997). High consumption of green leafy vegetables therefore plays vital role in human nutrition and health (Odukoya et al., 2007 and Aget et al., 2000).

According to Okechukwu et al. (2019), rats fed fresh sample extract of Cocoyam inflorescence showed significantly ($p<0.05$) higher hemoglobin count than those fed the control sample at all the doses of administration. Accessibility, affordability and with fewer side effects compared with synthetic drugs are some of the factors contributing to increased use of plant products in developing countries in the prevention or management of various metabolic disorders (Mowla et al., 2009).The protective effects of these plant foods are considered to be related to various phytochemicals contained in their vegetables. These protective effects of vegetables include their antioxidant activities (Okechukwu et al., 2019). As in fruits, vegetables also contain many antioxidants which help to protect the body from oxidative stress, disease and cancers (Merken and Beecher, 2000). Antioxidants help the body to develop the capacity to fight diseases by boosting the body's immunity (Okechukwu et al., 2019). This function of antioxidant in addition to health consciousness among other factors appears to have drawn research attention to the identification of biologically active components in foods that exhibit potentials to reduce the risk of diseases (Okechukwu et al., 2019).

Natural antioxidants play a key role in health maintenance and prevention of chronic and degenerative diseases, such as atherosclerosis, cardiac and cerebral ischema, carcinogenesis, neurodegenerative disorders, diabetic pregnancy, rheumatic disorder, DNA damage and ageing (Uddin et al., 2008; Jayasri et al., 2009). Odukoya et al. (2007); Mishra and Tumnay (2011) reported that high consumption of leafy vegetables has been associated with a lowered incidence of degenerative diseases. Green leafy vegetables are among the foods that play this dual role of providing nutrients and health benefits. Vegetables are harvested at all stages of maturity and used either as processed, semi- processed or fresh by man, but they are usually offered fresh to livestock. Cocoyam inflorescence is one of the vegetables which is cultivated in the tropics and subtropical region.

Cocoyam inflorescence has a stout peduncle, which is a little shorter than leaf stalks, and coloured with pale yellow color and the length is about 20 cm long (Okechukwu et al., 2019). It is consumed as delicacies in the southern part of Nigeria. The inflorescence of C. esculenta emerges from the center of the corm surrounded by the leaves (Okechukwu et al., 2019). There are four (4) species of colocasia esculenta that produce inflorescence, namely Nigerian colocasia esculenta (NCe) which include; NCe002 (Ede ofe green), NCe003 (Ede ofe Purple), NCe004 (Ede ofe giant/green) and NCe005 [Ede ofe light yellow] (Okechukwu et al., 2019). This NCe005 is locally called Ukpon (Anambe or inambe) (Okechukwu et al., 2019). Cocoyam inflorescence begins to emerge from cocoyam plant between July and September which really shows that it has matured for harvest (Okechukwu et al., 2019). Among all these only NCE005 is commonly consumed in the southern parts of Nigeria and has the highest yield during their season [Okechukwu et al., 2019 and Kalu et al., 2020]. This work is only channeled to NCE005 sample only.

Generally, processing improves the bioavailability of plant food proteins including vegetable proteins. Traditionally, fresh cocoyam inflorescence can be used fresh as a culinary vegetable. It is also dried, milled and used as flavourant in some communities to impart peculiar flavour. However, use of fresh cocoyam inflorescence is usually associated with irritating sensation in the mouth and throat due to the presence of oxalate (Kalu et al., 2020). Elimination of oxalate and other toxic substances in Cocoyam inflorescence through processing will enable gainful exploitation of other useful inherent components in this vegetable (Okechukwu et al., 2019). Many food processing techniques have been highlighted as possible means of reducing or totally eliminating antinutrients in plant foods (Okechukwu et al., 2019). However, there is paucity of information on the effects of boiling method on the anti-oxidant constituents of Cocoyam inflorescence.

Material and Methods

Sample Collection: Fresh samples of cocoyam (Colocasia esculenta (L.) schott) inflorescence NCe005 used for this study were obtained from cocoyam section of National Root Crop Research Institute (NRCRI) Umudike, Abia State where it was as well identified.

Sample Preparation: Fresh cocoyam inflorescence were washed, drained and allowed to air dried for about 40 minutes at a room temperature. The first portion of fresh sample was used as control. The fresh cocoyam inflorescence was sliced (2mm) and blended using Kenwood blender-BL300/BL350 series to obtain the wet milled fresh sample. It was packaged in a plastic container and stored in the freezer until used for analysis.

The second portion was boiled at 100°C for 10 min and allowed to cool and air dried. The boiled samples were sun dried for a period of five days. The dried sample was milled using Kenwood blender-BL300/BL350series. They were packaged in a plastic container and stored in a refrigerator (10°C) until used for analysis.

Analysis

Antioxidant Vitamins and minerals

Vitamin A, C, E and B2 were determined using HPLC as described by Junaid et al. (2008). Samples were determined by reversed- phase high performance liquid chromatography technique using Agilent 1100 series Model HPLC system equipped with degasser, quaternary pump, autosampler, UV detector and column (zornax SB C8, 4.6 x 75mm, and 3.5 µm particle size or X bridge C18, 4.6 x150mm, and 5µ particle size) in an isoelectric elution mode and at a constant flow rate of 1ml/min using Agilent pump. Stable operating LC conditions were established before HPLC analysis by equilibrating for 30 min with mobile phase (ca 1 ml/ min). Standard blanks respectively were injected before analysis to confim absence of chromatographic activity at retention time for the vitamins. The individual vitamin peaks in the samples was identified by comparison of retention times to the standards. The concentration of the vitamins in mg/100g edible weight was calculated.

Copper, iron and zinc were obtained when 1g of the sample was ashed in a furnace at 5500C for 3hrs. The ash was dissolved in 10MHCl (hydrochloric acid) in a conical flask and filtered into a 10ml flat bottomed standard flask and made up to the mark with distilled water. These individual minerals were measured from the solution using Atomic Absorption spectrophotometer (AAS) (UNICAM model 939 United Kingdom). Iodine was determined by the method of Muir and Lambert (1973).

The antioxidant properties were determined using the reducing power (RP) method as described by Odukoya et al. (2005). The concentration of the extract used was 25µg/mil. In this method, 1.0ml extract was mixed with 2.5ml of phosphate buffer (200mM, pH 6.6) and 2.5ml of potassium ferricyanide (30mM) and incubated at 500C for 20 mins. Thereafter, 2.5ml of trichloroacetic acid (600mM) was added to the reaction mixture, centrifuged for 10min at 3000rpm. The upper layer of solution (2.5ml) was mixed with 2.5ml of distilled water and 0.5ml of $FeCl_3$ (6mM) and absorbance was measured at 700nm. Ascorbic acid was used as the positive control.

Result and Discussions

Zinc content decreased significantly ($p<0.05$) in the processed samples. The fresh sample had 60.70 mg Zn content which is higher than 27.98 mg reported by Kalu (2009) for fresh Telfaria occidentalis leaf. In boiled samples Zn recorded 6.54mg. This lower Zn content observed in boiled, was attributed to leaching (Mepba et al., 2007 and Kawashima and Valente Soares, 2005). Reddy and Love (2014) noted that the bioavailability of minerals such as iron, zinc and calcium is known to be significantly affected by fibre, phytic acid and tannin content of foods. Negi and Roy (2001a) reported that the nutrients in vegetable generally decrease with boiling, soaking and blanching time. Drying method did not impact significant influence on the zinc content of cocoyam inflorescence. Boiling for 10min seemed to retain reasonable quantity of Zinc in cocoyam inflorescence. Recommended dietary allowance (RDA) for zinc is 1mg/day for men and 8 mg/day for women. This shows that both fresh and processed forms of cocoyam inflorescence are good sources of zinc. Consumption of cocoyam inflorescence can meet dietary requirement of Zinc.

The concentration of iron in the fresh sample was as low as 0.003mg. Mepba et al. (2007) noted that vegetables are generally poor sources of iron. There was a significant increase of Fe in the boiled samples. Boiled and sun dried sample showed significant increase in Fe from 0.003 to 1.12mg. This observed increase in iron content was due to loss of moisture during drying process. Drying method did not seem to exert any significant (p> 0.05) influence on the Fe content of the sample. However, Mbah et al. (2012) noted that sun dried Moringa oleifera showed Fe content of 0.73mg respectively. According to Amaefute and Obioha, (2001) boiling and dehydration processes impact on the essential nutrients in vegetables. Boiling seemed not to increase iron losses in cocoyam inflorescence. The iron content of this inflorescence especially the boiled and sun dried sample compared to an extent to the recommended dietary allowance (RDA) of 5.00-9.00 mg (WHO, 2001). Increase in consumption of both fresh and boiled cocoyam inflorescence may meet up our target iron level in the body system. Okechukwu et al., 2019 reported that at the middle dose of 200 mg/kg body weight, increases were observed in PCV, RBC and HB of animals that were administered fresh and

dried cocoyam inflorescence relative to the values shown at 100 mg/kg dose of administration in all the treatment due probably

Means along the column with different alphabetical superscript indicates a significance difference (P<0.05) at 5% level of significance. Each value represents the mean of the triplicate determination.

The copper content of cocoyam inflorescence follows the same trend as iron. The copper content of the fresh sample was 0.04 mg which was lower when compared with 4.33 mg/100g reported by krejpcio et al. (2007) for Ocimum basilicum (Basil). Boiled sample showed Cu content of less than 1mg/100g. The Cu values observed in this study are higher than 0.098 mg and 0.128 mg/100g reported by Mirian and Cosmos (2010) for curry leaf powder but lower than 10.11mg/100g reported by the same authors for rosemary leaves. The apparent increase in the copper content of the processed sample was attributed to moisture loss which increased the concentration of the minerals. Trace elements are usually needed by the body in a minute quantity. The level of Cu observed in this study for both fresh and processed sample is within the WHO limit for Cu in spices. The recommended dietary allowance (RDA) for copper for adult males and females is 0.9 mg/day, 1mg for pregnant women, 1.3 mg for lactating mothers and 10 mg as tolerable upper intake level (FNB, 2001). Therefore, the level of copper (Cu) in the samples are very tolerable and may not be harmful to the body system.

Vitamins

The vitamin content of cocoyam inflorescence is represented in Table 1. The results showed that boiing of Cocoyam inflorescence led to losses of vitamins. The vitamin C content of cocoyam inflorescence was 27.21mg suggesting that Cocoyam inflorescence is a good source of vitamin C. The vit C content obtained in this study compares with 27.44 mg/100g reported by Musa and Ogbadoyi (2012) for fresh Hibiscus sabdariffa and higher than 15.20 mg and 20.00 mg reported by Antia et al. (2010) and Muller, (1988) for fresh sweet potato leaves and orange respectively. The decrease observed by the boiled sample could be attributed to leaching, thermal destruction and oxidation (Blaring, 2006). Similar decreases in vit. C as a result of blanching, soaking and boiling of vegetables were reported by Nkafamiya et al. (2010). George (1999) and Gernah and Ajir (2007) reported that heat processing especially boiling can cause huge losses of vitamin C due to the thermo sensitive and hydrosoluble nature of the compound. The significant (p<0.05) losses of vitamin C could as well be as a result of sun drying used during the processing. Sun drying may not be a good method of processing that will conserve vitamin C in vegetables (Olaofe, 1999). Loss of ascorbic acid is used as an indicator of food quality, and the severity of treatment (Passmore and Eastwood, 1986). Processed cocoyam inflorescence cannot be depended as a sole dietary source of vitamin C.

The pro-vitamin A content of the fresh sample was 348.9 µg but boiling reduced the level significantly to 11.28 mg. Osum et al. (2013) reported a higher (3583.26 IU) value for Uchakoro leaf. The significant ($p<0.05$) decrease of pro-vit A in the boiled sample was attributed to thermal destruction and oxidation (Roch, 1990 and Fellows, 2000). Tindall's (1983) also noted decreases in pro-vitamin A and C contents of vegetables and increase in mineral content with drying. Retention of nutrients especially vitamins decrease with longer cooking time and higher cooking temperature. According to USDA, 1998 and Rickman et al., 2007 moderate boiling/cooking is preferable in other to conserve and improving the availability of beta-carotene in vegetables. According to Mulokozi and Svanberg (2003), provitamin A carotenes are highly reduced by open sun-drying. The RDA of vitamin A is 900 µg (5400 µg of carotene). The fresh sample is a better source of pro vitamin A than the boiled sample. Dependence on processed samples like sun drying method as is practiced traditionally will not satisfy the requirement for the vitamin.

Vitamin E content of fresh cocoyam inflorescence was 16.82 mg. This value is lower than 53.36mg reported by Osum et al. (2013) for raw uchakoro leaf. Boiling reduced the vitamin E content of the samples to 0.43 mg/100g. The level of vitamin E in boiled cocoyam inflorescence was low. The significant decrease of vit E in the processed samples was attributed to the effect of heat and oxidation that occured during processing. Ottaway (1993) reported that vitamin E is unstable to heat in the presence of air. Vitamin E is readily oxidized by air and decomposed by light. Vitamin E is reasonably stable to pH changes. The principal function of vitamin E is as an antioxidant. The recommended dietary allowance of vitamin E according to food and nutrient Board (2000) is 15 mg/day as against 10mg it used to be in 1989. Fresh cocoyam, inflorescence would be good source of vitamin E where it is needed. Fresh cocoyam inflorescence having contained 16.82 mg of vit E above the recommendation (15 mg) might be a good source of antioxidant since Vit. E has been shown to exhibit antioxidant properties. Okechukwu et al., 2019 reported that the rats fed fresh sample extract showed significantly ($p<0.05$) higher hemoglobin count than those fed the control sample at all the doses of administration which he suggested might be as a result of antioxidant phytochemicals present in the inflorescence. Nwankpa et al. (2014) and Sodipo et al. (2013) reported similar observation in rats fed Phyllanthus amarus and aqueous extract of Solanum macrocapus.

The vitamin B2 content of the fresh cocoyam inflorescence was 12.59 mg. This value is higher than 9.13mg vit B2 content reported by Osum et al (2013) for raw Uchakoro leaf. Boiling and sun drying treatment significantly decreased vit, B2 to 0.59 mg. This significant ($p<0.05$) decrease of vit B2 could be attributed to low pH, effect of heat and leaching. Vitamin B2 is unstable to light, alkaline condition and heat (Ottaway, 1993). Vitamin B2 is lightly soluble in water, sensitive to pH, light and excessive heat (Okaka et al., 2002 and Fellows, 2000). The recommended daily allowance (RDA) of vitamin B2 for adult human beings (14-19 years) range between1.7-3.4 mg/day (FNB, 1998). Below 74 years old can consume between 0.3-0.9 mg/day. The vitamin B2 content of both fresh and processed samples is appreciable thus cocoyam inflorescence can be said to be a good source of vitamin B2. Vitamin B2 has a powerful antioxidant activity from its role as a precursor to FMN and FAD (Dutta, 1993). Deficiency of vit B2 includes weakness, fatigue, mouth pain and tenderness, eye burning and itching. But advanced deficiency leads to cheilosis, angular stomatitis, dermatitis, cornea vascularization, anaemia, and brain dysfunction (Cimino et al., 1987). Therefore cocoyam inflorescence could be recommended to sick people especially, the psychiatrics, cancer patients, and malaria patience as food supplement or alternative source of vitamin B2. This also implies that both fresh and processed cocoyam inflorescence are good sources of antioxidant vitamins. This could be the reason why the roasted stem of cocoyam leaves or even the inflorescence is used in eastern part of Nigeria to treat injuries in between the toes of the human legs inflicted by Jegger contacted by swimming in a dirty running waters by placing the hot stem on the injured part of the body for a period of 10 minutes and the wound heals.

Antioxidant Property of Cocoyam Inflorescence

The antioxidant property of cocoyam inflorescence is shown in Table 2. The reducing power of the fresh samples was 0.663 and it differs significantly (p<0.05) from that of the control which was 0.529. The reducing power of the fresh sample compares with the value (0.530) reducing power reported by Yakubu et al. (2012) for fresh bitter leaf. The higher value of reducing power observed for the fresh cocoyam inflorescence compared to that in boiled sample suggests that most of the phytochemical constituents and antioxidant components responsible for the reduction activity are intact. There were significant decrease (p<0.05) in the reducing property exhited by the boiled sample (0.308). This significant (p<0.05) reduction in reducing power was also observed by Yakubu et al. (2012) who reported 0.460 and 0.510 reducing power for boiled and blanched samples of bitter leaves respectively. According to Mulokozi and Svanberg (2003) and Albinhn and Savage, (2001) pro vitamin A carotenes, nutrients and bioactive compounds were highly reduced by traditional sun drying. In this research, the fresh cocoyam inflorescence recorded the highest reducing property, higher than the control (citric acid). It is evident therefore, that cocoyam inflorescence exhibits anti-oxidant activities as other conventional herbs and spices and consequently should be used as other spices are used. Compounds with reducing power are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants.

In conclusion, the results of this research showed that cocoyam inflorescence consist of minerals and vitamins with high concentration of potent antioxidant that can be used as spices and food additives that can alleviate oxidative stress and related disorders.

Table 2: Antioxidant Property (By Reducing Power At 700nm)

Samples	Absorbance (700nm) 25µg/ml of the sample
Vitamin C (Control)	0.529±0.031a
Fresh sample	0.663±0.003b
Boiled sample	0.308c±0.003

Mean ±Standard Deviation. Mean down the column with different alphabetical superscript indicates significant difference (p<0.05). Each value represents the mean of the triplicate determination.