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Research Article

Amino Acid Content, Fatty Acid Profile and Radical Scavenging Capacities of *Coccinia grandis* (L.) Voigt. Fruits

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Abstract: In Sudan, the unripe (green) fruits of *Coccinia grandis* (L.) Voigt. (Cucurbitaceae) are eaten raw as salads or cooked by either boiling or frying in oil. In this study the amino acid content, fatty acid profile and radical scavenging capacities of the raw and boiled fruits were determined. The total amino acids ranged from 7736 mg/100 g in raw fruits to 7766 mg/100 g in boiled fruits and with the exception of leucine, boiling did not cause significant difference in the essential amino acids content (32.5%) of the fruits. Boiling caused significant ($p<0.05$) change in the amount of the total saturated (from 38.04% in raw fruits to 14.156% in boiled ones) and unsaturated fatty acids (from 57.59% in raw fruits to 85.774% in boiled ones). Moreover, boiling caused significant ($p<0.05$) losses of vitamin C by 73% and polyphenols by 70.6 % contents. The high antioxidant activity of the raw fruits (IC_{50} 22 mg/L), determined by DPPH and ABTS assays, was declined dramatically upon boiling. In conclusion, the results of this study suggested that fruits of *C. grandis* could have health beneficial effect and their consumption in the raw state is preferable.

Keywords: Antioxidant activity, *Coccinia grandis*, essential amino acids, polyunsaturated fatty acids, total phenolics, vitamin C

INTRODUCTION

Many studies recognized the potential of many different wild edible plants as food provides nutritional and health benefits (VanderJagt *et al*., 2000; Cook *et al*., 2000). In many parts of Africa, especially in rural communities, the use of wild edible plants as food source is an integral part of the culture of indigenous people (Bussmann *et al*., 2006; Grivetti and Ogle, 2000; Medley and Kalibo, 2007). Bhat and Karim (2009) stated that, the vast numbers of underutilized species represent an enormous resource which can help to meet the increasing demand for food and nutrition, energy, medicine and industrial needs.

Fruits and vegetables are considered to be the major contributors of nutritional antioxidants, which may decrease incidence of chronic diseases, such as cardiovascular disease, cancer, diabetes, Alzheimer's disease, cataracts and age-related functional decline in addition to other health benefits (Zhang and Hamauzu, 2004; Cao *et al*., 1996; Cohen *et al*., 2000; Knekt *et al*., 2002; Liu *et al*., 2000).

Sudan's flora is rich in wild plants which have good nutritional values. Until recently, little attention has been given to the role of wild edible plants in Sudan. *Coccinia grandis* (L.) Voigt. (Cucurbitaceae) ia a fast growing shrub or small tree found in tropical Africa and Asia. The plant was introduced by human mostly as food crop to several countries in Asia, Australia, Pacific Islands, Caribbean and Southern United States (Muniappan *et al*., 2009). In Sudan, *C. grandis* found in western Sudan as wild and also cultivated for its fruits. The unripe (green) fruits are eaten raw as salads or cooked by either boiling or frying in oil. However, thermal processing has long been known to cause significant losses of physical characteristics and chemical composition of vegetables (Sukhwant *et al*., 1992; Price *et al*., 1998; Khachik *et al*., 1992; Zhang and Hamauzu, 2004). Information regarding the nutritional content and beneficial effect of raw and boiled green *C. grandis* fruits is meager. As part of our on-going documentation on the nutritional potential of wild food plants from Sudan, the present study aims to analyze the amino acids content, fatty acids profile, vitamin C, total phenolics and radical scavenging capacities of raw and boiled unripe (green) *C. grandis* fruits.

MATERIALS AND METHODS

Plant materials: Green fruits of *C. grandis* were collected from Southern-West Kordofan in July 2011 and were identified. Voucher specimen (No. 1109KD7) was deposited in the Herbarium of Botany Department, Faculty of Science and university of Khartoum.

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Preparation of samples and extracts: Fruits were washed with tap water and were divided into two portions. One portion was retained raw, peeled, sliced and air dried. The other was cooked by steaming for 10 min, then peeled, sliced and air dried. Dry raw and processed samples were pounded and were kept at-20°C until analyses. All calculations were made according to dry matter basis.

Ethanolic extracts of raw and boiled samples were also prepared for total phenolic and antioxidant capacity determination. The ethanol extract was prepared by soaking 20 g of sample in 200 mL ethanol at ambient temperature for 6 hours. The extract was decanted, filtered and concentrated in a rotary evaporator to yield 1.4 and 1.2 g from raw and boiled fruits respectively.

Chemicals: Ninhydrin, boron trifluoride, metaphosphoric acid, dichloroindophenol indophenol sodium salt, Folin–Ciacalteu reagent, L-ascorbic acid, gallic acid, 1, 1-diphenyl-2- picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzthiazoline- 6-sulphonic acid) (ABTS), potassium persulfate were purchased from Sigma-Aldrich (France). Other chemicals used were all analytical grade.

Amino acids analysis: Amino acids composition of samples was measured as hydrolysate using an amino Acid Analyzer (Sykam-S7130) based on high performance liquid chromatography technique. Sample hydrolysis was prepared following the method of Moore and Stein (1963). 200 mg of sample were taken into a hydrolysis tube. 5 mL 6 N HCl were added to the sample. The tube was tightly closed and incubated at 110°C for 24 h. After incubation period, the solution was filtered and 200 µL of the filtrate were evaporated to dryness at 140°C for an hour. The hydrolysate was diluted with 1 mL of buffer (citrate buffer pH 2.2). Aliquot of 150 µL of sample hydrolysate was injected in cation separation column at 130°C. Ninhydrin solution and an eluent buffer (the buffer system composed of solvent A of pH 3.45 and solvent B of pH 10.85) were delivered simultaneously into a high temperature reactor coil (16 m length) at a flow rate of 0.7 mL/min. The buffer/ninhydrin mixture was heated at 130°C for 2 min to accelerate chemical reaction of amino acid with ninhydrin. The products of the reaction mixture were detected at wavelength of 570 nm (440 nm for proline) on a dual channel photometer. The amino acids were identified by their retention time and wavelength ratio calculated from the areas of standards obtained from the integrator and expressed as mg/100 g.

Fatty acids analysis: Fatty acid profiles of total lipids were determined after transesterification with 14% boron trifluoride in methanol (1:1 v/v). Fatty acid methyl esters were analyzed by GC–MS (QP 2010 Shimadzu GC-MS equipment). Supelco equity 1 column with a film thickness of 30 m x 0.25 microns was used. The total flow rate was 24 mL/min and column flow rate was 1 mL/min. Ultra high purity Helium was used as the carrier gas with injector split ratio of 20: 1. The ion source and inter-phase temperatures were 200 and 250°C, respectively. The solvent cut time of 4 min and detector gain was 0.70 kv. A Wiley 229 library search was conducted on major peaks of the sample in order to identify the components of the sample. The relative percentage of each compound was determined.

Determination of vitamin C: The modified method of Bahorun *et al*. (2004) was used to determine the vitamin C content of raw and boiled fruits of *C. grandis*. 10 g of sample was blended with 40 mL of a solution of 3% metaphosphoric acid in 8% glacial acetic acid, pH 1.5, for 1min. The extract was then mechanically shaken for 15min in darkness filtered through glass wool. After filtration the clear extract was stored at -40°C prior to analysis by the 2, 6-dichloroindophenol titrimetric method (AOAC, 1995). Triplicate titration was conducted for all samples.

Determination of total phenolics: Total phenol contents in the extracts of raw and boiled fruits of *C. grandis* were determined using modified Folin-Ciocalteu method (Wolfe *et al*., 2003). Ethanol extracts were resuspended in ethanol to make 50 mg/mL stock solutions. An aliquot of the extract was mixed with 5 mL Folin-Ciocalteu reagent (previously diluted with water at 1:10 v/v) and 4 mL (75 g/L) of sodium carbonate. The tubes were vortexed for 15 s and allowed to stand for 30 min at 40°C for color development. Absorbance was then measured at 765 nm using the SHIMADZU UV-2550 UV-VS spectrophotometer. Total phenolic contents were expressed as gallic acid equivalents (mg/100 g) using the following equation based on the calibration curve: y = 0.0057x, R2 = 0.9315, where x was the absorbance.

DPPH radical-scavenging test: Antioxidant activity of the extracts of raw and boiled fruits of *C. grandis* was estimated using DPPH *in vitro* method (Mensor *et al*., 2001). Test samples were dissolved separately in methanol to get test solution of 1 mg/mL. Series of extract solutions of different concentrations (1, 5, 10, 20, 40, 60, 80 and 100 µg/mL) were prepared by diluting with methanol. Assays were performed in 96-well, microtiter plates. 140 µL of 0.6×10-6 mol/L DPPH was added to each well containing 70 µL of sample. The mixture was shaken gently and left to stand for 30 min in dark at room temperature. The absorbance was measured spectrophotometrically at 517 nm using a microtiter plate reader (Synergy HT Biotek, logiciel GEN5). Blank was done in the same way using

methanol and sample without DPPH and control was done in the same way but using DPPH and methanol without sample. Ascorbic acids were used as reference antioxidant compound.

The ability to scavenge DPPH radical was calculated by the following equation:

$$\text{DPPH radical scavenging activity (\%)} = 1- [(Abs_{sample}–Abs_{blank})]/(Abs_{control})]\times100$$

where,
Abs_{sample} = The absorbance of DPPH radical+sample
Abs_{blank} = The absorbance of sample+methanol
$Abs_{control}$ = The absorbance of DPPH radical+methanol

The IC_{50} value was calculated from the linear regression of plots of concentration of the test sample against the mean percentage of the antioxidant activity. Results were expressed as mean±SEM and the IC_{50} values obtained from the regression plots (Sigma PlotsR 2001, SPSS Science) had a good coefficient of correlation, ($R^2 = 0.998$).

ABTS radical-scavenging test: A second *in vitro* method was performed to estimate antioxidant potential of the extracts: ABTS assay, based on the method of Re *et al.* (1999). Test samples were dissolved separately in methanol to get test solution of 1 mg/mL. Series of extract solutions of different concentrations (1, 5, 10, 20, 40, 60, 80 and 100 µg/mL) were prepared by diluting with methanol. The ABTS radical cation ($ABTS^{*+}$) was produced by reacting 7 mM stock solution of ABTS with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12 h before use. The obtained $ABTS^{*+}$ solution was diluted with methanol to an absorbance of 0.700±0.02 at 734 nm. 190 µL of $ABTS^{*+}$ solution was added to each well containing 10 µL of sample. The mixture was shaken gently and left to stand for 15 min in dark at room temperature. The absorbance was measured spectrophotometrically at 734 nm using a microtiter plate reader (Synergy HT Biotek®, logiciel GEN5). The $ABTS^{*+}$ scavenging capacity of the extract was compared with that of ascorbic acids and the percentage inhibition calculated as:

$$\text{ABTS radical scavenging activity (\%)} = [(Abs_{control}–Abs_{sample})]/(Abs_{control})]\times100$$

where,
$Abs_{control}$ = The absorbance of $ABTS^{*+}$ (= 0.700 ± 0.02)
Abs_{sample} = The absorbance of sample+$ABTS^{*+}$

The IC_{50} value was calculated from the linear regression of plots of concentration of the test sample against the mean percentage of the antioxidant activity. The IC_{50} values obtained from the regression plots (Sigma PlotsR 2001, SPSS Science) had a good coefficient of correlation, ($R^2 = 0.9926$).

Statistical analysis: All analyses were performed in triplicate and data reported as mean±Standard Deviation (SD). The Student's t-test was used to analyse differences between raw and boiled fruits. Results were processed by Excel (Microsoft Office 2010).

RESULTS AND DISCUSSION

Amino acids content: The composition and amount of amino acids in C. *grandis* raw and boiled fruits are presented in Table 1. The total amino acids ranged from 7736 mg/100 g in raw fruits to 7766 mg/100 g in boiled fruits where the essential amino acids represent 32 and 33%, respectively. With the exception of leucine, boiling did not cause significant difference in the essential amino acids content of the fruits. The most abundant component of essential amino acids, in decreasing order, were isoleucine ranged from 880 mg/100 g (44% of RDA) in boiled fruits to 864 mg/100 g (43.2% of RDA) in raw ones and leucine ranged from 744 mg/100 g (19% of RDA) in boiled fruits to 704 mg/100 g (18% of RDA) in raw ones. The major components of non-essential amino acid were alanine, ranged from 1424 mg/100 g in boiled fruits to 1632 mg/100 g in raw fruits and proline, ranged from 1520 mg/100 g in raw fruits to 1760 mg/100 g in boiled ones. Methionine was the limiting amino acid. Previous study (Getachew *et al*., 2013) on the amino acids content of *C. grandis* leaves from India showed that the leaves were rich in amino acids especially in lycine, which is the main limiting amino acid in cereal grains. However, in this study with the exception of isoleucine, the amount of all essential amino acids content in *C. grandis* fruits from Sudan was lower than that reported from the Indian *C. grandis* leaves (Getachew *et al*., 2013).

Table 1: Amino acids profile of the unripe (green) fruits of *Coccinia grandis* (dry weight basis, mg/100 g)

		C. grandis fruits		
Amino acids		Raw	Boiled	*t*-Test
Essential				
	Thr	200±13	208±8.0	
	Met	48±4.0	48±1.70	
	Ile	864±5.5	880±4.5	
	Leu	704±5.5	744±3.0	p<0.05
	Tyr+Phe	448±6.9	424±4.6	
	Lys	120±4.6	96±5.00	
	His	128±9.0	126±2.6	
	Total	2512	2526	
Non-essential				
	Asx	640±4.5	592 ± 6	p<0.05
	Ser	208±7.0	176 ± 2.6	p<0.05
	Glx	504±4.0	584 ± 4.5	
	Gly	96±5.00	80 ± 3.6	
	Ala	1632±14	1424 ± 31	p<0.05
	Arg	624±26	624 ± 3	
	Pro	1520±17	1760 ± 40	p<0.05
	Total	5224	5240	
Total amino acids		7736	7766	

ND: Not Detected; each value represents mean ± S.D. of triplicate (n = 3)

Table 2: Composition of fatty acids (dry weight basis, %) of the unripe (green) fruits of *Coccinia grandis)*

		C. grandis fruits		
Fatty acids		Raw	Boiled	*t*-Test
Caproic acid	C6:0	0.07±0.01	0.03±0.0200	p<0.05
Lauric acid	C12:0	0.10	0.05±0.0020	p<0.05
Tridecanoic acid	C13:0	ND	0.006±0.001	p<0.05
Myristoleic acid	C14:1	ND	0.014±0.002	p<0.05
Myristic acid	C14:0	0.41±0.02	0.63±0.0200	
Pentadecenoic acid	C15:1	0.15	0.22±0.0200	
Pentadecanoic acid	C15:0	0.72±0.02	0.70±0.0400	
Palmitoleic acid	C16:1	0.21±0.01	ND	p<0.05
Palmatic acid	C16:0	35.98±1.7	10.60±0.400	p<0.05
Heptadecanoic acid	C17:1	ND	33.81±0.800	p<0.05
Linolenic acid	C18:3	9.68±0.2	0.300±0.090	p<0.05
Linoleic acid	C18:2	0.39±0.04	ND	p<0.05
Linolelaidic acid	C18:2	15.3±0.4	16.84±0.50	
Oleic acid	C18:1	31.05±0.8	34.51±0.50	p<0.05
Stearic acid	C18:0	4.25±0.09	1.720±0.02	p<0.05
Eicosatrienoic acid	C20:3	0.10±0.01	ND	p<0.05
Eicosadienoic acid	C20:2	0.82±0.2	ND	p<0.05
Arachidonic acid	C20:4	0.03±0.01	ND	p<0.05
Heneicosanoic acid	C21:0	0.15±0.01	0.12±0.01	
Behenic acid	C22:0	0.52±0.01	0.26±0.02	p <0.05
Tricosanoic acid	C23:0	0.09±0.02	0.04±0.01	
Nervonic acid	C24:1	0.16±0.02	0.08±0.02	p<0.05
Total Saturated Fatty Acids (TSFAs)		38.04	14.156	
Total Unsaturated Fatty Acids (TUSFAs)		57.59	85.774	
Monounsaturated Fatty Acids (MUSFAs)		31.57	68.634	
Polyunsaturated Fatty Acids (PUSFAs)		26.02	17.14	

ND: Not Detected; each value represents mean ± S.D. of triplicate (n = 3)

Table 3: Vitamin C and total phenolic content of the unripe (green) fruits of *Coccinia grandis*

Fruit	Vitamin C (mg/kg)	Total phenolic content (mg GAE/100 g)
Raw	122.4±1.3	473±3.0
Boiled	33±0.3	139±1.0
t-Test	p<0.05	p<0.05

Each value represents mean ± S.D. of triplicate (n = 3)

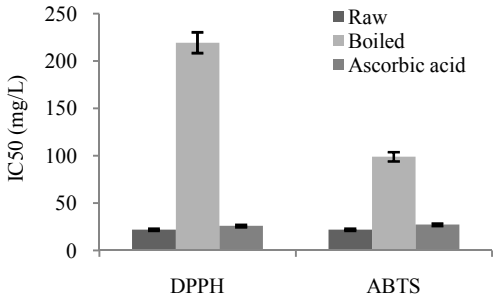

Fig. 1: Antioxidant activity of the unripe (green) fruits of *Coccinia grandis* (n = 3); the values are significantly different (p<0.05) when compared to the control

Fatty acids composition: The composition of fatty acids in raw and boiled *C. grandis* fruits is shown in Table 2. Boiling caused significant change (p<0.05) in the amount of the total saturated and unsaturated fatty acids. Total saturated fatty acid reduced from 38.04% in raw fruits to 14.156% in boiled ones, whereas, total unsaturated fatty acids increased from 57.59% in raw fruits to 85.774% in boiled ones. Monounsaturated Fatty Acids (MUSFAs) and polyunsaturated fatty acids (PUFAs) accounted for 31.57 and 26.02% in raw fruits and 68.634 and 17.14% in boiled ones respectively. Ratio of unsaturated fatty acid: saturated fatty acid was 1.51 in raw fruits and 6.06 in boiled ones. Palmatic acid (35.98%) and oleic acid (31.05%) represented the most abundant fatty acids in raw fruits while oleic acid (34.51%) and heptadecanoic (33.81%) were the predominant fatty acids in the boiled *C. grandis* fruits. Fatty acids especially the PUFAs of plant origin are known to play an important role in the management of coronary heart disease (Shajeela *et al*., 2013). Thus, the high level of unsaturated fatty acids in *C. grandis* fruits is nutritionally desirable.

Vitamin C and polyphenol content: A significant (p<0.05) decrease in vitamin C and polyphenol content was observed after boiling the fruits. Boiling led to a decrease of 73% in vitamin C content and 70.6 % in polyphenolic content (Table 3) suggesting that vitamin C and polyphenols could be largely leached into the cooking water. Some workers have reported losses of vitamins and phenolics from vegetables during cooking procedures (Price *et al*., 1998; Fennema, 1997). Moreover, Hunter and Fletcher (2002) have indicated that boiling processes at above 95°C would decompose the antioxidant components of vegetables.

Antioxidant activity: The antioxidant activity of the ethanolic extracts of raw and boiled *C. grandis* fruits was evaluated by DPPH radical and ABTS radical cation assays. The results showed that raw fruits had high DPPH and ABTS radicals scavenging capacity (IC_{50} 22 mg/L) comparable to that of ascorbic acid (IC_{50} 27 mg/L) (Fig. 1). However, boiling caused sharp decrease in the antioxidant activity of the fruits

with IC_{50} 219.338 and 99.108 mg/L from DPPH and ABTS assays respectively. This decrease in antioxidant activity could be correlated to the decrease in both vitamin C and total phenolic content after boiling. Moreover, this result agrees with earlier reports on the effect of cooking on the antioxidant properties of many vegetables (Ismail *et al*., 2004, Zhang and Hamauzu, 2004, Xu and Chang, 2008). Rawson *et al*. (2013) reported that, thermal processing may have varying effect on the bioactive phytochemicals in fennel bulbs due to the oxidation, dehydrogenation, or loss of water molecules leading to a more stable compound formation which may be less bioactive in most cases.

CONCLUSION

In conclusion, the results of this study suggested that fruits of *C. grandis* could have beneficial effect for food and/or nutricentical application in the promotion of health. Consumption of the fruits in their raw state is recommended as results of this study indicated that antioxidant activity declined, along with antioxidant components, during the cooking.

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