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Assessment of elemental content in the fruit of Graviola plant, *Annona Muricata*, from some selected communities in Ghana by instrumental neutron activation analysis

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ABSTRACT

The major (K, Mg, Cl, Ca and Na) and minor (Mn, Fe, Cr, Co, Cu and Br) elemental concentrations in different parts of Graviola (*Annona Muricata*) fruit including the fruit fibres, fruit cover, seed, fruit juice and seed cover were determined using instrumental neutron activation analysis. Their concentrations were found to vary in the various fruit parts. The elements Br, Ca, Cl, K, Mg, Mn and Na were recorded in all the various fruit parts. The highest concentrations recorded were K in the fruit cover (1.43±0.03%), fruit fibre (1.46±0.09%), fruit juice (2.28±0.15%) and seed (0.55±0.06%). In the seed cover however, Ca recorded the highest concentration of 0.25±0.02%. Chromium was below the detection limits of INAA in the fruit cover, fruit fibre and fruit juice. Copper and Fe were below detection limits in the fruit juice and seed cover respectively. The presence of Ca, Mg, Na, K, Co, Cr, Cu, Fe, Br, Cl and Mn reflects the function of the Graviola fruit as a source of essential nutrient elements. Therefore, the Graviola fruit becomes important in view of the fact that their regular consumption might help the body to attain the required levels of these essential nutrient elements.

Introduction

Fruits are nutrient suppliers, acting on the metabolism of several functions in humans, and their main nutrients (minerals and vitamins) influence the performance of these functions. Hence, several researchers (Kiran *et al* 2011; Tufuor *et al* 2011 and Mahammad *et al*. 2010) have conducted studies on the mineral composition of different fruits.

Graviola (*Annona Muricata*) belonging to the family Annonaceae is a small, evergreen tree that produces heart-shaped, edible fruit widely consumed by a majority of people in Ghana. The bark, leaves, roots, fruit, and fruit seeds of the Graviola contains the chemical compound, Annonaceous Acetogenins, which has remarkable cytotoxic, anti-tumoural activity.

The research on Graviola has identified more than 40 compounds with anti-cancerous properties, capable of killing cancer cells (Kojima, 2004 and Yuan et al 2003). It is also known for its antimicrobial actions, antiparasitic, antimalarial and insecticidal actions (Takahashi et al, 2006, Gbeassor et al, 1990).

Human growth and metabolism rely on a balanced diet, which includes vitamins and inorganic micronutrients. Although constituting a small fraction of the whole diet, inorganic micronutrients play an important role in various metabolic processes and their deficiency or excess may disturb the normal biochemical function of the body (Adotey *et al*, 2009). The elements considered essential are the major elements (sodium, magnesium, phosphorus, chloride, potassium and calcium) and the minor elements (chromium, manganese, iron, cobalt, copper, zinc, selenium, molybdenum and iodine) (Crews, 1998). Due to awareness of the need for a balanced diet by most Ghanaians, coupled with the fact that Graviola (*Annona Muricata*) has several medicinal application, the aim of this Ghanaians, coupled with the fact that Graviola (Annona Muricata) has several medicinal application, the aim of this study was to determine the major (K, Mg, Cl, Ca and Na) and minor elemental (Mn, Fe, Cr, Co, Cu, Se, Mo, Zn and Br) content in the various parts of Graviola (*Annona Muricata*) fruit using instrumental neutron activation analysis.

Methodology

Sample preparation

Graviola fruit samples were randomly purchased from some selected major markets within the Greater Accra region of Ghana for analysis. The samples were placed in pre - cleaned polyethylene bags and transported to the laboratory.

In the laboratory, ten sub-samples from each market were selected and thoroughly washed with doubly distilled water. The fruit covers (epicarp) of the sub-samples were peeled off into pre-cleaned polyethylene bags. Their fruit juices were then squeezed into pre cleaned polyethylene containers leaving behind the fruit fibres and seeds. The seeds were then isolated from the fibres. Using stainless steel knife, the seeds were subsequently separated into seed cover (seed coat) and seed (endosperm). The epicarp, fruit fibres, endosperm and seed coat were frozen at -20°C and lyophilized using a Christ Gamma 1-16 for 72 hrs at -30°C corresponding to a vapour pressure of 0.370 mbar.According to Hoeing, 2001, drying by lyophilization ensures initial sample texture preservation and also facilitates subsequent milling of samples. The dried fibres, seed and seed

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cover were then homogenized separately using a commercial blender with stainless steel blades.

About 200 mg each of the pulverized samples (i.e. dried fibres, epicarp, fruit fibres, endosperm and seed coat) and the fruit juice were weighed, wrapped and heat sealed in ultra-clean polyethylene films. Five replicate sub-samples were prepared from each sample. The method described by Sarfo *et al.,* 2011, was used for packaging of the samples for short, long and medium lived radioisotopes irradiation. Standard reference materials namely IAEA-V-10 (Hay powder) and NIST–SRM 1572 (Citrus Leaves) were prepared and packed similarly as the fruit samples. However, for the medium and long lived radioisotopes, the standard reference materials were sandwiched between four wrapped samples and together, packaged into one polyethylene vial for irradiation.

Sample Irradiation and counting

The method used for irradiation and counting of samples have been described previously by Serfor-Armah, 2006. The prepared samples, standards and empty polyethylene vials were all irradiated in the Ghana Research Reactor-1(GHARR-1) facility at the Ghana Atomic Energy Commission, Kwabenya, operating at 15 kW at a thermal neutron flux of $5x10^{11}$ $ncm^{-2}s^{-1}$. Samples were transferred into the irradiation sites via pneumatic transfer system at a pressure of 0.6 MPa. The categorization of irradiation was done based on the half-life of the elements of interest. Irradiation time (ti), decay time (td) and counting time (tc) for short-lived radionuclides with half-life less than few hours (i.e. ^{80}Br, ^{49}Ca, ^{38}Cl, ^{66}Cu, ^{56}Mn and ^{27}Mg) were 2 minutes, 1-10 minutes and 10 minutes respectively. For medium-lived radionuclides with half-life of several hours (^{42}K and ^{24}Na), the irradiation time used was one hour, decay time twenty-four hours and a counting time of one hour. The long lived radionuclides with half-life in days and years (i.e. ^{60}Co, ^{51}Cr and ^{59}Fe) were irradiated for four hours and decayed for two weeks with ten hours counting. In short irradiation, each of the sealed samples in the polyethylene capsules were sent for irradiation one after the other in one of the inner irradiation channels. Table 1 describes the nuclear data for the elements of interest.

Evaluation of peak area of γ-spectrum

The counting of the induced radioactivity was performed by a PC- based γ-ray spectrometry. It consists of an n-type high purity Germanium (HPGe) detector (model GR2518) coupled to a computer-based Multichannel Analyzer via electronic modules and a spectroscopy amplifier (model 2020, Canberra Industries Incorporated). The relative efficiency of the detector is 25% with an energy resolution of 1.8 keV at γ-ray energy of 1332 keV of ^{60}Co.

The γ-ray product radionuclides were qualitatively identified by the energies emitted and the quantitative analysis was done by converting the counts as area under the photo peaks by the comparator method. Through appropriate choice of cooling time, the detector's dead time was controlled to be less than 10 %. Both qualitative and quantitative analyses were done using the γ-ray spectrum analysis software, MAESTRO-32.

Results and discussion

NIST–SRM 1572 (Citrus Leaves) and IAEA-V-10 (Hay powder) were irradiated and counted. This was to evaluate the reliability of measurements in terms of accuracy and precision using the irradiation schemes employed.

The precision (expressed as % relative standard deviation) of the methods were obtained for the five replicates and was within ±4%. Using the Pearson's correlation, it was observed that a positive correlation existed between the results obtained from this work and the certified/recommended values from the issuing agencies. From these observations, it can be concluded that a good agreement existed between the results from this work and that of the reported values from the issuing agencies. The results from the two reference materials are shown in Table 2.

The average concentration of Br, Ca, Cl, Cr, Co, Cu, Fe, K, Mg, Mn and Na analyzed in the epicarp, Fruit fibre, Fruit juice, edosperm and Seed coat of Graviola (*Annona Muricata*) using INAA are given in Table 3 in µg/g dry weight of the samples (unless indicated otherwise). Each result is an average of at least three independent measurements with a precision of about ± 1%.

From the results in Table 3, the highest concentrations recorded were K in the epicarp (1.43±0.03%), fruit fibre (1.46±0.09%), fruit juice (2.28±0.15%) and edosperm (0.55±0.06%). In the seed coat however, Ca recorded the highest concentration of 0.25±0.02%. Chromium was below the detection limits of INAA in the fruit cover, fruit fibre and fruit juice. Though Cr was below detection limits in the epicarp, fruit fibre and fruit juice, it recorded a concentration of 0.87± 0.13 µg/g and 0.55± 0.08 µg/g in the edosperm and seed coat respectively. Copper and Fe were below detection limits in the fruit juice and seed coat respectively. The presence of the detected metals in the samples is an indication that Graviola fruit may be used to compensate for the deficient intake of such essential elements through other diets.

The concentration of Br in the various fruit parts in increasing order was 3.13±0.77 µg/g (epicarp), 4.27±2.40 µg/g (Seed coat), 5.59±0.84 µg/g (edosperm), 6.29±2.31 µg/g (Fruit juice), 40.40±9.77 µg/g (Fruit fibre). Though Br is one of the inorganic elements that have not been established yet as essential, it is known to contribute to biological processes (Macrae *et al,*1993).

In the epicarp, fruit juice and edosperm, Co recorded concentrations of 0.06±0.009 µg/g , 0.40±0.06 µg/g and 0.02±0.004 µg/g respectively. The concentration of Co was more pronounced in the fruit juice than the other fruit parts. Chromium was found in detectable amounts in the edosperm (at a concentration of 0.87±0.13 µg/g) and seed coat at a concentration of 0.55±0.08 µg/g. The concentration of Cu in the samples in decreasing order was 48.20±11.78 µg/g (fruit fibre), 22.90±5.86 µg/g (endosperm), 11.96±0.89 µg/g (epicarp) and 0.53±0.08 µg/g (seed coat). Cobolt, Cr and Cu (Valkovic, 1975) are essential for hair growth and for increasing the rate of milk production by pregnant female (Ahmad et al 2008; Abbasi *et al.,* 2009). Copper, in appropriate concentrations, aids hormone synthesis and protein metabolism (Bakhru, H.K, 2002). A daily dietary intake of 2 to 3 mg of copper is recommended for human adults (Dara, 1993). However, ingestion of 15-75 mg of copper causes gastrointestinal disorders.

Though Fe was detected in all the samples analysed, the highest concentration of 121.51±18.23 µg/g was recorded in the epicarp. The lowest concentration of Fe (i.e 13.34±10.15 µg/g) was also detected in the fruit fibres. Iron recorded concentrations, in µg/g, of 80.99±10.15 and17.86 ± 2.68 in the fruit juice and the endosperm respectively. According to an estimate, 57.6% of the body iron in human is contained in hemoglobin and 8.9% in myoglobin, whereas approximately 33% in non-heme iron complexes, including ferritin and haemosiderin. The cytochrome enzyme contain about 0.5% of iron (Jacob & Worwood 1974). The importance of ensuring adequate bio-available dietary Fe arises from the severe consequences associated with iron deficiency and anemia, including reduced immune function and resistance to infection, development delays, irreversible work performances and adverse frequency outcomes (Cooper *et al.* 2006; Naghii and Fouladi, 2006). Therefore, the graviola fruit becomes important in view of the fact that its regular consumption might elevate iron level in the body.

Calcium, Cl, K and Mg were the major elements recorded in the various fruit parts analysed (i.e had their concentrations greater than 100 µg/g). Concentrations of Ca ranged from 0.36±0.02% (in endosperm) to 0.24±0.02% (in the fruit juice) whiles Cl ranged from 0.41±0.007% (in the epicarp) to 0.03±0.002% (in the seed coat). Though the concentration of K ranged from 2.28±0.14% in the fruit juice to 0.02±0.004% in the seed coat, that of Mg ranged from 0.15±0.013% (i.e edosperm) to 0.02±0.006% (i.e seed coat). Concentrations of 0.14±0.03%, 0.11±0.02% and 0.15±0.02% were however recorded for Mg in the epicarp, fruit fibre and fruit juice respectively. Magnesium is an important electrolyte responsible for proper nerve and muscle function. It also works as co-factor in many metabolic reactions (Yamashita *et al*, 2005). Calcium is a metal known to be essential for blood clotting and muscle contraction. It also plays a role in the formation and maintenance of bones. Potassium aids in the fluid balance and nerve impulse transmission within the cells (Witney and Rolfes, 2005).

The highest concentration of Mn (17.33±1.26 µg/g) was recorded in the endosperm with a minimum concentration of 2.99±0.45 µg/g found in the fruit juice. Concentrations of 14.54±2.90 µg/g, 8.61±1.36 µg/g and 9.18±1.02 µg/g were recorded in the epicarp, fruit fibre and seed coat respectively. Manganese is essential for normal functioning of nerve, heartbeat, central nervous system and a good anti-oxidant. It is a micronutrient for bone formation and aids in enzymatic actions (Bakhru, 2002). A daily dietary intake of 2.5 to 5 mg of manganese by human contributes to the well being of the cells (Dara, 1993). Manganese deficiency causes diseases and excess of it causes poisoning of the central nervous system.

The concentrations of Na recorded were in the order 310.53±22.58 (fruit juice) > 248.76±17.73 (seed coat) > 232.55±38.03 (epicarp) > 143.15±15.89 (endosperm) > 104.47±15.99 (fruit fibre).

The presence of Ca, Mg, Na, K, Co, Cr, Cu, Fe and Mn reflects the function of the Graviola fruit as a source of essential nutrient elements, which often act as co-factor activators in metal-ligand enzyme complexes (Valkovic, 1975)

Trace elements are present in human body in very low amount, usually less than 1 micro-organism per gram of the tissues (Lawrence *et al*., 1993), some elements are essential trace elements and some are not essential but have well defined evidence in human metabolism.

Iron is an essential trace element for human metabolism and has recommended dietary allowance (RDA) of 10 to 15 mg. Copper, Mn and Cr also have evidence of essentiality in human metabolism and have an RDA of 1.5 to 3 mg, 2.5 to 5.0 mg and 50 to 200 mcg respectively. An estimated safe dietary daily intake limits have been established (Lawrence *et al*., 1993) for Cu, Mn and Cr. The comparison of recommended dietary allowance and safe daily dietary intake of trace elements with our analyzed values of trace elements are given in Table 4.

Conclusions

The fruit parts of Graviola, *Annona Muricata*, was analysed for their essential elements and was found that the fruit fibres contained the essential elements Ca, Cl, Cu, Fe, K, Mg and Mn. The seed cover contained Ca, Cl, Cr, Cu, K, Mg and Mn with the seed containing the elements Ca, Cl, Cr, Co, Cu, Fe, K, Mg and Mn in detectable concentrations. The fruit cover of Graviola (*Annona Muricata*) fruit was found to contain Ca, Cl, Co, Cu, Fe, K, Mg and Mn with the fruit juice containing Br, Ca, Cl, Co, Fe, K, Mg, Mn and Na. The elements Br, Ca, Cl, K, Mg, Mn and Na were recorded in all the various fruit parts. The highest concentrations recorded were K in the fruit cover (1.43±0.03%), fruit fibre (1.46±0.09%), fruit juice (2.28±0.15%) and seed (0.55±0.06%).

The results of this study indicate that the fruit studied is well endowed with essential nutrients required for humans. The presence of high elemental concentrations in the Graviola fruit gives a new insight into their potential use as a medicinal plant and to compensate for element deficiencies in man and animals.

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Table 1: Nuclear data used for determination of elemental concentrations (IAEA TECDOC-564)

Element	Nuclear Reaction	Half life	Gamma ray energy (Kev)
Br	$^{79}Br(n, \gamma)\,^{80}Br$	17.7 min	616.2
Ca	$^{48}Ca(n, \gamma)\,^{49}Ca$	8.7 min	3084.4
Cl	$^{37}Cl(n, \gamma)\,^{38}Cl$	37.3 min	1642.4, 2167.5
Cr	$^{50}Cr(n, \gamma)\,^{51}Cr$	27.72 d	320.0
Co	$^{59}Co(n, \gamma)\,^{60}Co$	5.27 y	1173.2, 1332.4
Cu	$^{65}Cu(n, \gamma)\,^{66}Cu$	5.1 min	1039.4
Fe	$^{58}Fe(n, \gamma)\,^{59}Fe$	44.5 d	1099.2, 1291.6
K	$^{41}K(n, \gamma)\,^{42}K$	12.36 h	1524.7
Mg	$^{26}Mg(n, \gamma)\,^{27}Mg$	9.46 min	843.8, 1014.4
Mn	$^{55}Mn(n, \gamma)\,^{56}Mn$	2.58 h	846.7, 1810.7, 2112
Na	$^{23}Na(n, \gamma)\,^{24}Na$	15.02 h	1368.6, 2754.1

Table 2: Comparison of elemental concentrations in reference materials analyzed by INAA with certified/recommended values, n = 5

Elements	NIST–SRM 1572 (Citrus Leaves) Concentration (µg/g unless indicated otherwise)		IAEA-V-10 (Hay powder) Concentration (µg/g unless indicated otherwise)	
	This work	Certified value	This work	Certified value
Br			7.00±1.05	8.00±1.20
Ca%	3.20±1.01	3.15± 0.10	2.15±0.32	2.16±0.32
Cl	414.11±20.71	414.00*		
Cr	0.79±0.01	0.80±0.20	6.20±0.21	6.50±0.90
Co			0.13±0.02	0.13±0.002
Cu	17.00±0.82	16.50±1.00	9.00±0.68	9.40±3.05
Fe	90.04±12.00	90.00±10.00	190.01±20.03	186.00±18.51
K%	1.81±0.02	1.82 ± 0.06%	2.00±0.30	2.10±0.02
Mg%	0.55±0.03	0.58 ± 0.03%	0.14±0.02	0.13±0.02
Mn	23.50±3.21	23.00±2.00	45.50±6.83	47.00±2.11
Na	160.10±18.50	160.00±20.00	50.20±7.53	50.00±4.52

*Recommended value or uncertified value

Table 3: Essential elements content (in µg/g unless otherwise indicated) in the fruit parts of graviola

Minerals	Fruit cover	Fruit fibre	Fruit juice	Seed	Seed cover
Br	3.14±0.77	40.40±9.77	6.29±2.31	5.59±0.84	4.27±2.40
Ca%	0.29±0.03	0.30±0.02	0.24±0.017	0.36± 0.02	0.25±0.02
Cl%	0.41±0.007	0.15±0.004	0.18±0.003	0.03± 0.002	0.02 ±0.002
Cr				0.87±0.13	0.55±0.08
Co	0.06±0.009		0.40±0.06	0.02±0.004	
Cu	11.95±0.89	48.20±11.78		22.90± 5.85	0.53±0.08
Fe	121.51±18.23	13.34±10.15	80.99±10.15	17.85±1.34	
K%	1.43±0.03	1.46±0.09	2.28±0.15	0.55± 0.06	0.02±0.004
Mg%	0.14±0.03	0.12±0.02	0.15±0.02	0.15 ±0.013	0.02±0.006
Mn	14.54±2.91	8.61±1.36	2.99±0.45	17.33± 1.26	9.17±1.02
Na	232.55±38.03	104.47±15.99	310.53±22.58	143.16± 15.89	248.70±17.72

Table 4: Recommended dietary allowance and estimated safe daily dietary intake of trace elements for human beings (Lawrence *et al*., 1993).

Trace element	Maximum Conc. of trace elements observed in some parts of the graviola fruit (µg/g)					Recommended dietary allowance (RDA)	Estimated safe adequate daily dietary intake
	Fruit cover	Fruit fibre	Fruit juice	Seed	Seed cover		
Fe	121.51± 18.23	13.34± 10.15	80.99± 10.15	17.85± 1.34		10–15 mg	
Cu	11.95±0.89	48.20± 11.78		22.90± 5.85	0.53± 0.08	1.5 to 3 mg	0.9 mg
Mn	14.54± 2.91	8.61± 1.36	2.99± 0.45	17.33± 1.26	9.17± 1.02	2.5 to 5.0 mg	11 mg
Cr				0.87±0.13	0.55±0.08	50 to 200 mcg	25-35 µg