Study of Proximate, mineral and anti-nutrient composition of *Punica granatum* seeds from North-Western Nigeria, and Saudi Arabia

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ABSTRACT: The seeds of Punica granatum fruit from Nigeria and Saudi Arabia were analyzed for nutritional and anti-nutritional composition using standard analytical methods. The results obtained were studied. The results obtained show that there were significant differences ($p<0.05$) in percentage ash, moisture, crude lipid, crude protein, crude fiber, available carbohydrate and the energy value .Elemental analyses show that magnesium is the most abundant elements in the seeds of both countries Nigeria and Saudi Arabia (5650mg/100g) and (1140.1mg/100g) respectively, this may be due to its availability soil where it was grown, while copper and zinc are the least abundant. The result of anti-nutritional analyses indicate significance difference ($p<0.05$) in hydrocyanic acid, nitrate, soluble oxalate, phytate and tannin. The anti-nutrients to nutrients ratio indicate the availability of some minerals. The study show that differences and similarities in nutritive contents with respect to Protein, Fiber, Fat / Lipids, Calcium, Iron magnesium, Sodium, Potassium, etc in the two samples may be due to climatic condition, genetic factor, mode of cultivation, topography and other factors. Considering the nutritional and anti-nutritional profile of the two samples, it can be conclude that the seed is a very good source of some nutrients which if utilized properly would assist in providing some of these nutrients to the people in the society; this may assist in combating the problem of malnutrition in Nigeria and Africa in general.

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INTRODUCTION

The relevance of fruits and the need to introduce more plant food in order to bridge the gap of alarming food shortage in human nutrition have aroused attention of various researchers throughout the world especially Nigeria into evaluation of nutritional and anti-nutritional status of various fruits (Anhwange et al., 2004; Hassan et al., 2004; Umar et al., 2007). Malnutrition is a major health problem in Africa, despite government's efforts to promote food production. Protein-energy malnutrition in infants and children is one of the most common nutritional problems. (Achu, 2004). Developing nations are devastated by hunger and diseases even though the nations have abundant fruits and other natural resources which if properly used could solve these problems. (Vainio - Maltila, 2000). Some diseases such as diabetes, obesity, cancer, and cardiovascular diseases are manifesting in developing countries than ever before; and are due to inadequate consumption of fruits and vegetables. If this trend continues, world Health organization (WHO) projected that, the percentage of people living with diabetes alone in developing countries will rise by 170% by the year 2025 (Ganry, 2008). Human beings required food to grow, reproduce and maintain good health without food, our bodies could not stay warm, build or repair tissues or maintain a heartbeat. Eating the right foods can help us void certain diseases or recover faster when illness occurs. These and other important functions are fueled by chemical substances in our food called nutrients (Worthington – Robberts, 2008).

Punica granatum, commonly known as "*pomegranate" (Hausa-Rumann*), borrowed from Arabic name of the plant. Kingdom: *plantae*, division: *magnohophyta,* class: *magnohopsida*, subclass: *Rosidae,* order: *myrtales*, family: *Lythraceace,* Genus: *Punica*, species: *P. granatum*, Bionomial name: *Punica granatum L.*, synonyms; *Punica Malus* (Morton, 1987).

The edible fruit is a berry and is between a lemon and a grape fruit in size, 5-12 cm in diameter with a rounded hexagonal shape, and has thick reddish skin and around 600 seeds. Each seed has a surrounding water-laden pulp-the aril-ranging in color from white to deep red or purple. This aril is the edible part of the fruit. The seeds are embedded in a white, spongy, astringent pulp (Wikipedia, 2011).

Researchers have shown that seeds not only contain nutritionally important, but are also sources of other phyto-chemicals which have anti-nutritional effects.

MATERIALS AND METHODS

Sample collection

Punica granatum fruits were randomly plucked from different trees at Dadin Kowa Area, and Federal College of Education, both in Kontagora metropoly, Kontagora Local Government Area of Niger State, Nigeria.

The sites were chosen because of the abundance of this plant. The fruits were collected from different branches of the selected trees as described in the method of (Ayaz et al., 2002), and (Asaolu and Asaolu, 2002). The foreign (Saudi Arabia) sample was obtained from Bab sheriff market, Jedda and Othaim shop of Hafral-baten both in the Kingdom of Saudi Arabia. The samples were then transported to the laboratory in paper bags.

The fresh fruits were authenticated at the Herbarium unit of the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto.

The two samples were treated in the same way but saparately.The sample was mixed thoroughly washed with distilled water to avoid surface contamination (Ahmed and Birnin Yauri, 2008). 1kg representative sample was obtained using alternate shovel method (Alam, 1996). The seeds were separated manually by squeezing ripe and good fruits. The seeds were air dried and grounded to fine powder using pestle and mortar. The oil was then extracted using soxghlet extraction method and n-hexane as solvent and then kept in airtight fiber containers in refrigerator until they were analyzed. (Nordeide et al., 1996).

RESULTS AND DISCUSSION

Proximate Analysis

Moisture content was determined at 105^{o}C in an oven. Ash content was determined at 550^{o}C.Crude protein, lipid, and fiber were determined according to the procedures of AOAC (1990),Crude Nitrogen was determined using the Kjeldahl procedure and crude protein value was obtained by multiplying the nitrogen value by a factor of 6.25, while estimation of available carbohydrate was done by difference according to equation (1).

CHO = 100 – (%ash+ %crude protein+%crude lipid +%crude fiber)……(1)

Energy kcal = [(%CHOx4)+(%Crude protein x 4) +(Crude lipid x 9)] (Hassan et al.,2008)

Mineral Analysis

The mineral elements were analyzed using walinga et al. (1989) method; the elements in the sample were brought into solution by wet digestion technique using a mixture of concentrated nitric, perchloric and sulphuric acids in the ratio 9:2:1 respectively. Fe, Zn, Ca, Mg, Cu, Mn, were determined by AAS, while Na and K were determined using atomic emission spectrometer and phosphorus was determined using colorimetric method.

Anti-nutritional Analysis

Oxalate was determined by the method of Krishna and Ranjhna (1980), while phytate and hydrocyanic acid were determined by the AOAC (1990) method. Nitrate was determined by IITA (1988) method.

Table 1. Proximate content of *Punica granatum f*ruits seeds from Nigeria and Saudi Arabia.

COMPONENT ANALYZED	NIGERIA	SAUDI ARABIA
Moisture (%WW)	48.40± 0.10	57.83± 5.77
Ash (%DW)	2.00± 0.10	1.53± 0.06
Crude lipid (%DW)	9.27± 0.06	5.10± 0.10
Crude protein (%DW)	2.59± 0.10	2.59± 0.10
Crude fiber (%DW)	3.96± 0.04	2.10± 0.10
Available carbohydrate (%DW)	39.54± 0.06	21.51± 0.06
Vitamin C	19.0± 0.06	20.6± 0.01
Energy value (kcal per 100g)	339.90± 0.10	219.46± 0.10

The data are mean value ± standard deviation of triplicate results.DW= dry weight and WW=wet wet

Table 2. Anti-nutritive content of *punica granatum f*ruits seeds from Nigeria and Saudi Arabia.

ANTINUTRITIVE FACTORS	NIGERIA	SAUDI ARABIA
Phytate	0.46± 0.06	1.60± 0.09
Cyanide	0.08± 0.06	0.28± 0.12
Nitrate	2.36± 0.06	2.52± 0.10
Tannin	13.66± 0.06	23.1± 0.10
Saponin	6.0± 0.06	5.0± 0.11
Soluble oxalate	3.15± 0.12	6.75± 0.10
Total oxalate	3.60± 0.10	9.45± 0.10

The data are mean value ± standard deviation of triplicate results

Table 3. Minerals content of *Punica granatum f*ruits seeds from Nigeria and Saudi Arabia

ELEMENT ANALYZED (mg/100g)	NIGERIA	SAUDI ARABIA
Fe	43.6± 0.10	21.0± 0.06
Zn	16.2± 0.06	5.70± 0.10
Mg	5650.0± 0.10	1140.1± 0.06
Ca	23.8± 0.06	44.00± 0.06
Cu	14.70± 0.06	11.00± 0.10
Na	47.50± 0.10	52.60± 0.10
K	155.0± 0.06	151.00± 0.06
P	55.2± 0.10	60.0± 0.06

The data are mean value ± standard deviation of triplicate results

Table 4 Anti-nutrient to nutrient molar ratio of Seed of *Punica granatum* obtained from Nigeria and Saudi Arabia

ANTI NUTRIENT TO NUTRIENT RATIO	NIGERIA	SAUDI ARABIA	CRITICAL LEVEL
(oxalate) / (ca)	$5.9x10^{-2}$	$4.9x10^{-2}$	2.5
(oxalate) / (ca+mg)	$1.7x10^{-3}$	$3.0x10^{-3}$	2.5
(ca) (Phytate) / (Zn)	$5x10^{-3}$	$5.7x10^{-3}$	0.5
(Phytate) / (Zn)	$3x10^{-3}$	$2.6x10^{-2}$	1.5
(Phytate) / (ca)	$3.6x10^{-3}$	$9x10^{-4}$	0.2
(Phytate) / (Fe)	$4.6x10^{-2}$	$6.3x10^{-2}$	0.4

Proximate Composition:

The result for proximate composition of the two samples are presented in Table 1.There were significant differences ($p<0.05$) in percentage moisture content, ash content, crude protein, crude lipid, crude fiber, carbohydrate and vitamin C, across the two samples. Generally the moisture content of Nigerian sample is 48.40% indicating that the sample has low moisture content compared to that of Saudi Arabia sample 57.83%.The result is different from those reported by other researchers Morton (1987) on juice and seed of the fruits to be 72.6-86.4%.Mahammad et al. (2010) reported 52.3% on seeds, pulp and peel of pears fruits. The low moisture content signifies the higher dry matter yield as reported by Bamigboye et al. (2010).The low moisture content do not favour growth and increase of microorganisms, but reflects on their long storage. The percentage ash content of Nigerian sample significantly differed ($p<0.05$) from that of Saudi Arabia. As indicated in Table 1, Nigerian sample has the highest value of 2.00% while Saudi Arabia sample has1.53%.The highest value reported not similar to that reported by other researchers 0.36-0.73% on the same fruit. Morton (1987), but similar to the value reported by Olaposi and Adunni (2010) that vegetables of *Cnidoscolus chayamansa ,Solanium nodiflorum* and *Senecio biafrae* has the ash content of 1.57,2.67 and 2.01 respectively. The crude lipid content of Saudi Arabia and Nigerian samples are within the range of 5.10 to 9.27%. These results are higher than those reported by Mele (2007) and Morton (1987), 2.41 – 3.73% and trace - 0.9% respectively. These results are lower than those reported by Osman (2004) that *Adansonia digitata* had crude lipid of 18.4%.The low lipid content which justifies the general observation that, fruits and vegetable are not good sources of fats and oils (Hassan et al.,2002).Although it might be relegated as source of oil commercially. Lipid are essential because they provide the body with maximum energy (Dreon et al., 1990).The crude protein content of the two samples are the same 2.59 %.These value are higher than the value reported by Morton (1987) 0.05-1.6%.The values are closer to that of *Solanium nodiflorum* 3.1%.The result show that the seeds contain some quantity of protein but at a very low concentration, it can serve as a source of protein considering the level of protein deficiency in the society. The highest percentage of carbohydrates was found in Nigerian sample (39.54%), while that of Saudi Arabia is (21.51%).These result are higher than 15.4-19.6% as reported by Morton (1987).these result suggest that *Punica granatum* seeds can also be a source of carbohydrate.

Vitamin C (ascorbic acid):

The levels of vitamin C in all the samples are shown in table 1.There was significant difference ($p<0.05$) in vitamin C among the sample. The result show that Saudi Arabia sample has the highest level of vitamin C (20.6 mg/100g) while that of Nigeria has (19,0mg/100g).These values are higher than the values reported by Morton (1987) which is 4-

4.2mg/100g, but lower than the value 1,050-312mg/100g as reported by Mele (2007) .FNIC (2011) recorded higher values of vitamin C in commonly used fruits such as mango (57.3mg/100g), pineapple (78.9mg/100g),Orange (69.7mg/100g), papaya (86.5 mg/100g) and strawberry (84.7 mg/100g).However FNIC (2011) indicate a closer values of other fruits including tomato (15.6mg/100g), watermelon (23.2mg/100g).

Mineral nutrients composition:

The results on mineral nutrients composition are indicated in Table 3.There was a significant difference ($p<0.05$) in mineral composition of Nigeria and Saudi Arabia samples. As indicated in Table 2. A healthy adult should eat less than 2400 mg of sodium per day to reduce the risk of elevated blood pressure because, on the average, the higher an individual's salt intake the higher his blood pressure. Keeping blood pressure in the normal range reduces an individual's risk of coronary heart diseases, congested heart failure, stroke and kidney diseases (NRC, 1989).

The result obtained in this study show that the seed is a better source of Mg, Ca, K, and P.

Magnesium and potassium are the most abundant elements found in the seeds of the two samples, the values of magnesium in the two samples Nigeria and Saudi Arabia are 5650.0± 0.10 and 1141.0± 0.06 mg/100g, these values closer to that of mele (2007), 1760-4270 mg/100g.The values of potassium in the two samples Nigeria and Saudi Arabia are 155.0± 0.06 and 151.00± 0.06 mg/100g, these values are lower than 259mg/100g obtained by other researchers (Morton, 1987).Magnesium plays an major role in relaxing muscle along the airway to the lungs thus, allowing asthma patients to breathe easier. It plays important roles in most reactions involving phosphate transfer (Appel, 1999). High amount of potassium in the body was reported to increase Iron utilization (Adeyeye, 2002) and beneficial to people taking diuretics to control hypertension and suffer from excessive excretion of potassium through the body fluid (Arinathan, et al., 2003).

Iron content of the two samples from Nigeria and Saudi Arabia is 43.6± 0.10 and 21.0± 0.06mg/100g.Iron is said to be important element in the diet of pregnant women, nursing mothers, infants convulsing patients and elderly to prevent anaemia and other related diseases (Oluyemi et al., 2006).

The Calcium concentration of the seeds from the two countries Nigeria and Saudi Arabia are 23.8± 0.06 and 44.00± 0.06mg/100g, these values are higher that reported by other researchers 3-12mg/100g (morton 1987).Calcium helps in regulating muscle contraction. It is also required by children, pregnant and lactating women for bones and teeth development (Margaret and Vickery, 1997).

The concentration of Copper in the fruit seed from the two countries Nigeria and Saudi Arabia is 14.70± 0.06 and 11.00± 0.10 mg/100g.copper deficiency has been reported to cause cardiovascular disorders as well as anaemia, bone disorder and nervous systems (Mielcarz et al., 1997).

Results of this analysis shows that the concentration of zinc in the seeds of the two countries Nigeria and Saudi Arabia are 16.2± 0.06 and 5.70± 0.10 mg/100g, Zinc is said to be an essential trace element for protein and nucleic acid synthesis and normal body development during periods of rapid growth such as infancy and recovery of illness (Melaku, 2005).

The levels of anti-nutritional factors are shown in Table 3.The results showed that tannins were highest in the entire samples. The sample from Saudi Arabia had tannins levels of 23.1±0.10mg/100g as the highest value whereas the Nigerian sample has 13.66±0.06mg/100g.These results suggest that, all levels of anti-nutritional determined in the samples are all below the recommended toxic levels cause by the presence of anti-nutritional factors(Brigitta and Caroline, 2000).However, even though the tannin level in the samples are slightly higher may be cause by analysis from the raw samples and can be reduced by cooking processes. Many studies have shown that tannin is carcinogenic and excessive ingestion of tannin from one or more sources, over a prolonged period, is detrimental to health (Morton, 1987).

The phytate content of the two samples Nigeria and Saudi Arabia are 0.46±0.06 and 1.60±0.09.Phytate in food bind some essential mineral nutrients in the digestive tract and can result in mineral deficiencies (Bello et al., 2008). The phytate composition of the samples are lower and might not pose any health hazard when compared to a phytate diet of 10-60 mg/100g which if consumed over a long period of time decreases bioavailability of minerals in monogastric animals (Thompson, 1993).Anti-nutrient to nutrients ratio were calculated in order to predict the bioavailability of some divalent elements (Mg, Ca, Fe and Zn).

To predict the effect of phytate on the bioavailability of Ca, Fe and Zn.Phytate to nutrients ratios were calculated. It shows that [phytate] / [Ca] ratio in the seed of the two samples Nigeria and Saudi Arabia was below the critical level of 0.2 like-wise the ratio [phytate] / [Zn] of both countries Nigeria and Saudi Arabia are ($3x10^{-3}$) and ($2.6x10^{-2}$) are also below the critical level of 1.5.

But [Ca][phytate][Zn] is a better measure of zinc bioavailability over [phytate][Zn] ratio (Obah and Amusa, 2009).The [Ca][Phytate][Zn] ratio in the samples of the two countries Nigeria and Saudi Arabia are ($5x10^{-3}$) and ($5.7x10^{-3}$), these values are below the critical level of 0.5, this shows that the bioavailability of Ca, Fe, and Zn may not be effected by the phytate content of the seed of *Punica granatum* fruit.

The concentration of nitrate in the two samples Nigeria and Saudi Arabia are 2.36±0.06 and 2.52±0.10 respectively. These values are within the acceptable daily intake 0f 3.7 mg/kg body weight (WHO).Higher intake of high nitrate concentrated food can lead to a disease called methemoglobinemia which is commonly known to reduce the ability of red blood cells to carry oxygen (Kim-Shapiro, et al., 2005).

High level of saponnin has been associated with gastroenteritis manifested by diarrhea and dysentery (Awe and Sodipo, 2001), but it was reported that saponnin reduces body cholesterol by preventing its reabsorpsion and suppresses rumen protozoan by reacting with cholesterol in the protozoan cell membrane thereby causing it to lyse (Umaru et al., 2007).The level of saponnin is within the acceptable level, the value of saponnin in the two countries Nigeria and Saudi Arabia are (6.0±0.06) and (5.0±0.11) respectively.

The concentration of hydrocyanic acid in the two samples Nigeria and Saudi Arabia are 0.08±0.06 and 0.28±0.12 respectively .These values are within the accepted level of human consumption. Only plant with more than 200mg of hydrocyanic acid equivalent per 100mg fresh weight are considered dangerous (Betancur-Ancona et al., 2008).

CONCLUSION

Results from the study have clearly shown that the two fruits of *Punica granatum* (sour type) from the two countries Nigeria and Saudi Arabia contained considerable amounts of carbohydrate, vitamin C and minerals which are vital in supplementing nutrition to mankind. It can therefore, be concluded from the study that differences and similarities in nutritive contents with respect to Protein, Fiber, Fat / Lipids, Calcium, Iron magnesium, Sodium, Potassium, etc in the two samples may be due to climatic condition, genetic factor, mode of cultivation, topography and other factors. Considering the nutritional and anti-nutritional profile of the two samples,

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