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Variation in nutrients composition of *Tetrapleura tetraptera* fruit at two maturity stages

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Abstract

Variation in the nutrients composition of the seed and pod of *Tetrapleura tetraptera* fruit was investigated at two maturity stages, namely, mature-green (MG) and ripe-brown (RB) stages. The proximate composition and food energy content on dry weight basis (DW) of the seed and pod varied significantly ($P < 0.05$) at the two maturity stages. MG seed (MGS) recorded the highest moisture content (6.87%); RB pod (RBP) had the highest carbohydrate (87.49 %), while RB seed (RBS) had the highest crude fat (14.46%), protein (28.72%), ash (5.59%) and food energy (424.60 cal/g). Starch, sugar, amylose and amylopectin also varied significantly ($P < 0.05$) in the seed and pod at the two maturity stages, such that MG pod (MGP) had the highest starch (48.19%); RBP had the highest sugar (31.71%) and amylopectin (99.90%), whereas MGS had the highest amylose contents (0.41%). There was also significant variation ($P < 0.05$) in the minerals levels (DW) of the seed and pod, with RBS having the highest Ca (15508.67 mg/Kg), Mg (403.54 mg/Kg), P (1494.74 mg/Kg), Fe (22.65 mg/Kg), Zn (5.25 mg/Kg) and Mn (59.35 mg/Kg). RBP had the highest Na (201.64 mg/Kg), K (4314.34 mg/Kg) and Co (1.16 mg/Kg), while MGS recorded the highest Se (1.21 mg/Kg) and Cu (8.25 mg/Kg) contents. Although advancing maturity had effect on the levels of the various nutrients of both the seed and pod of *T. tetraptera* fruit, generally the seed could provide more nutrients than the pod at the two stages of maturity investigated.

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Introduction

Nutrients both macro- and micro- play an important role in the maintenance of the body's well-being and metabolism. A balanced intake of nutrients (proteins, carbohydrates, fats, minerals and vitamins) helps in maintaining good state of health. Dietary proteins functionally promote growth, and are needed for the synthesis of enzymes, hormones and antibodies (Cheesebrough, 1987). Carbohydrates and fats provide the energy need of the body for physical, physiological and metabolic activities. Minerals serve a wide variety of essential physiological functions, ranging from structural components of body tissues to essential components of many enzymes and other biologically important molecules (Flynn, 1992).

Fruits constitute a significant component of the human diet. Fruit tissues development and maturation is the final phase of floral development that is signaled by successful pollination (O'Neill, 1997). Ripening is known to affect both the physical and chemical attributes of fruits, imparting numerous quality and nutritional characteristics upon the fruits (Giovannoni, 2001). Ripening influences various critical aspects of mature fruit, including fiber content and composition, lipid metabolism, and the levels of vitamins and various antioxidants (Ronen *et al.*, 1999). Factors such as climate, soil type and fertility, season, leaf-stem ratio, and physiology affect the nutrient composition of plants at different stages of maturity and development.

Tetrapleura tetraptera is a deciduous plant belonging to the mimosaceae family. It is generally found in the lowland forest of tropical Africa. In Nigeria, the tree begins flowering towards the end of February and is over in early April. The indehiscent pods are ripe from September to December, during which it is deciduous (Opabode *et al.*, 2011). The fruits consist of a fleshy pulp with small, brownish-black seeds. The fruits are green when tender and dark brown when fully ripe, and possess both nutritional and medicinal values (Adetunji and Aladesanmi, 2006). When dry, the fruit has a pleasant aroma and hence is used as a seasoning spice in the Southern part of Nigeria (Essien *et al.*, 1994; Aladesanmi, 2007). The dry fruit is also used in flavoring traditional pepper soup for breastfeeding mothers from the first day of delivery to prevent postpartum contractions, and to serve as a lactation aid (Nwaiwu *et al.*, 1986, Ojewole and Adewunmi, 2004). In West Africa this plant is used in ethnomedicine for the treatment of several ailments such as diabetes mellitus, hypertension, arthritis, asthma, epilepsy and schistomiasis (Ojewole and Adewunmi, 2004).

In view of the food and therapeutic uses of *T. tetraptera* fruit and the possible influence of maturity on its nutrients composition, this study was designed to evaluate the variation in the chemical composition and mineral content of the seed and pod of *T. tetraptera* fruit at two maturity stages.

Materials and methods

Samples collection and preparation

Triplicate samples of *T. tetraptera* fruit were randomly harvested from the plant at Idi-Ose village in Ibadan, Nigeria, at two maturity stages of the fruit development, and were authenticated at the Department of Botany, University of Ibadan, Nigeria. The first stage was at the MG stage (seven months after fruiting, September, 2012), while the second was at the RB stage (nine months after fruiting, November, 2012). The fruit samples were then rinsed with de-ionized water, after which the pods were manually separated from the seeds using a stainless steel knife. The samples were oven-dried at 50°C for 48 hours to a constant weight. Dried seed and pod samples (MGS, RBS, MGP and RBP) were subsequently milled to a fine particle size (0.5 mm) using a stainless steel milling machine, packed in air-tight plastic vials and stored at 4°C until analysis.

All the chemicals used for analysis were of analytical grade.

Determination of proximate composition and food energy

The proximate composition of the samples was determined according to methods of AOAC (2000).

Moisture content was determined by oven-drying at 100°C - 105°C for 18-24 h. Total Nitrogen content (N) was determined by Kjeldahl method, and the protein content was calculated as N x 6.25. Ash content was determined by incinerating 2 g of samples in a pre-weighed porcelain crucible in a muffle furnace at 600°C for 6 hours. Crude fat (ether extract) content of the samples was determined using a Soxtec extraction machine. Total carbohydrates were estimated as the difference of 100 and the sum of ash, protein, moisture and fat (i.e. 100 – [%ash + %protein + %moisture + %fat]).

The gross food energy was estimated according to the methods of Osborne and Voogt (1978) using the equation:

FE = (%CP x 4) + (%CHO x 4) + (%Fat x 9)

Where:

FE = Food energy (calories/g), CP = Crude protein, CHO = carbohydrates.

Determination of starch and soluble sugar content

Starch and total sugar were extracted from samples with 80% hot ethanol, and then quantified using phenol-sulphuric acid method as reported by Onitilo *et al.*, (2007).

Determination of Amylose and amylopectin content

0.1g of samples was dissolved in 1 ml, 95% ethanol and 9.2 ml, 1 N NaOH at 100°C in a water bath. After cooling, the solution was made up to 100 ml in a volumetric flask. Then an aliquot of 5 ml of the solution was added with 1 ml of 1 N acetic acid, and 2 ml iodine solution (0.2% I_2 in 2% KI) and the volume was made up to 100 ml with distilled water and mixed. After 20 minutes, the absorbance was measured at 620 nm using blank with 5 ml 0.09 N NaOH, 1 ml acetic acid and 2 ml iodine solution and made to 100 ml in total volume (Juliano *et al.*, 1981). Amylose content (%) was subsequently calculated from a standard curve prepared using amylose standard.

Amylopectin was calculated by difference (Juan *et al.*, 2006) using the following formula:

Amylopectin (%) = 100% – amylose (%).

Analysis of minerals

The minerals composition of the samples was determined according to the method reported by Shahidi *et al.*, (1999). 2 g of finely milled samples was subjected to dry ashing in an acid-washed porcelain crucible at 600°C in a muffle furnace. The resultant ash was dissolved in 5 ml of $HNO_3/HCl/H_2O$ (1:2:3) and heated gently on a hot plate until brown fumes disappeared. To the remaining material in each crucible, 5 ml of deionized water was added and heated until a colourless solution was obtained. The mineral solution in each crucible was transferred into a 100 ml volumetric flask by filtration through a whatman No 42 filter paper and the volume was made to the mark with deionized water. This solution was used for mineral analysis by atomic absorption spectrophotometer (model: A Analyst 200; Perkim Elmer, USA). Calibration curves of the different minerals analyzed were generated by first analyzing standard solutions of the individual minerals before samples analysis.

Sodium (Na) and potassium (K) were determined using flame photometer.

Phosphorus content of the digest was determined colorimetrically according to the method described by Nahapetian and Bassiri (1975). To 0.5 ml of the diluted digest, 4 ml of deionized water, 3 ml of 0.75M H_2SO_4, 0.4 ml of 10% $(NH4)_6MO_7O_{24}.4H_2O$ and 0.4 ml of 2% (w/v) ascorbic acid were added and mixed. The solution was allowed to stand for 20 min and absorbance readings were recorded at 660 nm. The phosphorus content of sample was subsequently calculated.

Statistical Analysis

Results of triplicate experiments were expressed as mean ± standard deviation (SD). Analysis of variance (ANOVA) and least significance difference (LSD) were carried out on the result data at 95% confidence level using SPSS statistical software package, version 17.

Results and discussion

The proximate composition (g/100 g DW) and food energy content (cal/g DW) of *T. tetraptera* seed and pod at the two maturity stages investigated are presented in Table 1. The result showed that the proximate composition of the seed and pod varied significantly ($P < 0.05$) at the two stages of maturity. The moisture content was in order of MGS > RBS > RBP > MGP, revealing that at the two maturity stages, the seed had higher moisture levels than the pod. This could be due to reduced transpiration in the seeds, since they are contained within the pod. The fat, crude protein, ash and food energy contents varied consistently in the order of RBS > MGS > MGP > RBP. This shows that whereas there was an increase in the fat, crude protein and ash contents of the seed from the mature-green stage to the ripe-brown stage, the levels of these nutrients decreased in the pod from the mature-green stage to the ripe-brown stage. This is an indication that the biosynthesis of fat, protein and most minerals continued in the seed of *T. tetraptera* during ripening, unlike in the pod where ripening may have been associated with the degradation these nutrients. A possible reason for the higher levels of these nutrients in the RBS relative to the MGS could be as a result of reduced activity of the enzymes degrading these primary metabolites which may have helped the seed in accumulating these nutrients during the later stage of ripening (Stanley, 1998). Conversely, the decrease in fat, crude protein and ash contents of pod with advancing maturity may be attributed to continued biosynthesis of secondary metabolites in response to external stimuli using these nutrients as precursors. The seed at both stages of maturity had higher levels of fat, crude protein, ash and food energy than the pod, implying that it has the potential to nourish the body more with these nutrients than the pod. Yu *et al.*, (2004) reported that the concentrations of nutrients in plant tissues vary with maturity stage.

Table 1. Proximate composition (g/100 g DW) and Food Energy content (cal/g DW) of *T. tetraptera* fruit at two maturity stages.

Nutrient	MGS	RBS	MGP	RBP
Moisture	6.87 ± 0.03^{a}	6.58 ± 0.02^{b}	4.07 ± 0.02^{d}	5.04 ± 0.01^{c}
Fat	7.50 ± 0.03^{b}	14.46 ± 0.04^{a}	1.71 ± 0.03^{c}	0.80 ± 0.01^{d}
Crude protein	21.17 ± 0.25^{b}	28.72 ± 0.26^{a}	4.45 ± 0.01^{c}	3.83 ± 0.01^{d}
Ash	5.35 ± 0.03^{b}	5.59 ± 0.02^{a}	3.62 ± 0.01^{c}	2.84 ± 0.02^{d}
Carbohydrate	58.89 ± 0.23^{c}	44.91 ± 0.28^{d}	86.16 ± 0.03^{b}	87.49 ± 0.01^{a}
Food energy	387.69 ± 0.18^{b}	424.60 ± 0.23^{a}	377.83 ± 0.16^{c}	372.48 ± 0.07^{d}
Starch	33.78 ± 0.31^{c}	25.05 ± 0.45^{d}	48.19 ± 0.31^{a}	36.73 ± 0.61^{b}
Sugar	5.74 ± 0.13^{d}	8.57 ± 0.23^{c}	13.52 ± 0.21^{b}	31.71 ± 0.30^{a}
Amylose	0.41 ± 0.01^{a}	0.20 ± 0.01^{c}	0.31 ± 0.01^{b}	0.11 ± 0.01^{d}
Amylopectin	99.60 ± 0.01^{d}	99.80 ± 0.01^{b}	99.70 ± 0.01^{c}	99.90 ± 0.01^{a}

Data represent the mean ± standard deviation of triplicate readings. Values with the same lowercase superscript letter along the same row are not significantly different ($P>0.05$).

Proteins are known to provide structural material for the human body and function as enzymes, hormones, and antibodies. Dietary proteins are the major source of the essential amino acids for humans (Hounsome *et al.*, 2008). Plant storage proteins such as lectins are involved in several biological processes, including antimicrobial/antiviral action, and in the inhibition of growth of tumor cells (De Souza Candido *et al.*, 2011).

Lipids (fat) in addition to providing fuel for metabolism are major components of cell membranes. Some plant lipids contain bioactive polyunsaturated fatty acids (omega 3 and omega 6) that have reported to be beneficial in preventing cardiovascular diseases, and decreasing the incorporation of cholesterol in the membranes of arteries (Vrablík *et al.*, 2009).

At the two stages of maturity studied, the pod had higher total carbohydrate, starch and soluble sugar levels than the seed, with RBP having the highest total carbohydrate and soluble sugar levels, and MGP having the highest total starch content. This shows that the pod is a better storage tissue for carbohydrates synthesized through photosynthesis. While the starch content of the seed and pod decreased with increasing fruit development (that is, ripening), the sugar level increased. The biosynthesis and degradation of starch have been reported to be closely associated with fruit development and commercial quality formation (Zhang and Wang, 2001). This observation could be attributed to increase in hydrolysis of starch due to higher activity of hydrolytic enzymes such as such as α-amylase, β-amylase and starch phosphorylase during ripening (Stanley, 1998), thereby leading to reduced starch level but increased sugar content. This finding is in agreement with the reports of other researchers (Pruthi, 1963; Pandya and Ramana Rao, 2010). Carbohydrates (sugars and starches) play the primary role of providing energy to body cells, particularly the brain, which is the only carbohydrate-dependent organ in the body. Sugars are involved in control of blood glucose and insulin metabolism, intestinal microflora activity, and food fermentation. Monosaccharides bound to protein and lipid molecules (glycoproteins and glycolipids) are involved in cell signaling (Hounsome *et al.*, 2008).

Whereas MGS had the highest amylose content, RPB had the highest amylopectin. At both stages of maturity, amylopectin was greater than amylose in both the seed and the pod. This observation is in consonant with that of Yotsawimonwat *et al.*, (2008) who reported that amylopectin is the major component in most starch.

Table 2. Minerals composition of *T. tetraptera* fruit (mg/Kg DW) at two maturity stages.

Mineral	MGS	RBS	MGP	RBP
Ca	13424.96 ± 9.67 ^{b}	15508.67 ± 20.91 ^{a}	2097.75 ± 4.09 ^{d}	3621.80 ± 7.32 ^{c}
Na	171.27 ± 0.13 ^{c}	170.67 ± 0.23 ^{c}	174.13 ± 0.34 ^{b}	201.64 ± 0.40 ^{a}
K	3810.20 ± 2.75 ^{d}	3362.50 ± 4.53 ^{c}	3873.61 ± 7.56 ^{b}	4314.34 ± 8.72 ^{a}
Mg	393.25 ± 0.28 ^{b}	403.54 ± 0.54 ^{a}	152.31 ± 0.30 ^{d}	218.94 ± 0.45 ^{c}
P	1455.09 ± 1.05 ^{b}	1494.74 ± 2.02 ^{a}	814.30 ± 1.59 ^{c}	721.18 ± 1.46 ^{d}
Fe	21.83 ± 0.01 ^{b}	22.65 ± 0.04 ^{a}	7.92 ± 0.02 ^{d}	11.63 ± 0.02 ^{c}
Zn	4.62 ± 0.01 ^{b}	5.25 ± 0.01 ^{a}	1.21 ± 0.01 ^{d}	1.42 ± 0.01 ^{c}
Mn	48.77 ± 0.04 ^{b}	59.35 ± 0.08 ^{a}	8.16 ± 0.02 ^{d}	22.69 ± 0.04 ^{c}
Se	1.21 ± 0.01 ^{a}	0.72 ± 0.01 ^{c}	0.73 ± 0.01 ^{c}	0.86 ± 0.01 ^{b}
Cu	8.25 ± 0.01 ^{a}	7.39 ± 0.01 ^{b}	4.32 ± 0.01 ^{c}	4.26 ± 0.01 ^{d}
Co	0.98 ± 0.01 ^{b}	0.96 ± 0.01 ^{b}	0.97 ± 0.01 ^{b}	1.16 ± 0.01 ^{a}
Na/K	0.04	0.05	0.04	0.05

Data represent the mean ± standard deviation of triplicate readings. Values with the same lowercase superscript letter along the same row are not significantly different (P>0.05).

Table 2 presents the minerals composition of the seed and pod of *T. tetraptera* fruit (expressed in mg/Kg DW). Minerals are essential elements, needed for the maintenance of good health. All the minerals analyzed except Co varied significantly ($P < 0.05$) in the seed and pod at the two stages of maturity investigated. Generally, the seed had higher levels of minerals than the pod, with RBS having the highest Ca (15508.67 mg/Kg), Mg (403.54 mg/Kg), P (1494.74 mg/Kg), Fe (22.65 mg/Kg), Zn (5.25 mg/Kg) and Mn (59.35 mg/Kg), followed by MGS. The preponderance of Ca in the RBS and RBP over MGS and MGP respectively is in agreement with the report of Poovaiah and Reddy (1987) who observed an increase in Ca level in seed and fruit with advancing development. Calcium plays an important role in plant growth and development, as it is required for both cell division and cell elongation. In human, it is required for muscle contraction, bone and teeth formation and blood clotting (Ahmed and Chaudhary, 2009; Peters and Martini, 2010). Phosphorus is related to calcium for bones, teeth and muscles growth and maintenance (Dosunmu, 1997).

Fig. 1a. Mature-green *T. tetraptera* seed.

Magnesium serves as a catalyst in energy producing reactions within the cells and facilitates muscle relaxation; it is also required for Ca metabolism in bones and management of circulatory diseases such as ischemic heart disease (George and Pamplana-Roger, 2004). Iron is an essential microelement for haemoglobin formation, normal functioning of the central nervous system and in the oxidation of carbohydrate, protein and fats (Adeye and Otokiti, 1999). Zinc serves as a co-factor for a large number of enzymes participating in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids as well as in the metabolism of other micronutrients. It also plays a central role in the immune system, where it affects a number of aspects of cellular and humoral immunity (Shankar and Prasad, 1998). Manganese is required in the formation of bones, for the metabolism of fats and carbohydrates and for fertility (Tolonen, 1990). Superoxide dismutase and the glycosyl transferase required for the biosynthesis of glycoproteins are also Mn-dependent enzymes (Robinson, 1987).

Fig. 1b. Ripe-brown *T. tetraptera* seed.

Whereas RBP had the highest Na (201.64 mg/Kg), K (4314.34 mg/Kg) and Co (1.16 mg/Kg) levels followed by MGP, MGS recorded the highest Se (1.21 mg/Kg) and Cu (8.25 mg/Kg) contents. This is an indication that at both stages of maturity, the pod of *T. tetraptera* fruit could provide more Na and K to the body than the seed. Sodium and potassium present in the intracellular and extracellular fluid help in maintaining the body's electrolyte balance and membrane fluidity (Ahmed and Chaudhary, 2009), and are important in maintaining proper nerves transmission in the body (Adeyeye 2002). Interestingly, the potassium content of both the seed and pod of *T. tetraptera* fruit obtained in this study at the two stages of maturity investigated were greater than their sodium contents; this variation is of significant importance particularly to a hypertensive patient (Vadivel and Janardhanan, 2000). To prevent high blood pressure, a Na/K ratio of less than one has been recommended (FND, 2002). In this study, the Na/K ratios obtained were 0.04, 0.05, 0.04 and 0.05 for MGS, RBS, MGP and RBP respectively. These ratios being in agreement with the Food and Nutrition Board (FND) recommendation, make *T. tetraptera* fruit good for maintenance of proper blood pressure.

Fig. 1c. Mature-green *T. tetraptera* Pod.

Selenium is a component of glutathione peroxidase (Arthur *et al.*, 1996) and thioredoxin reductase (Howie *et al.*, 1998**)**. These selenium-containing enzymes (selenoenzymes) are involved in controlling tissue concentrations of highly reactive oxygen-containing metabolites. During stress, infection, or tissue injury, these selenoenzymes may protect against the damaging effects of hydrogen peroxide or oxygen-rich free radicals (Arthur *et al.*, 1996). Cobalt is an essential trace element due its function as component of vitamin B12 (cyanocobalamin), whereas Cu is needed for hemoglobin and collagen production, healthy functioning of the heart, energy production, absorption of iron from digestive tract (Gupta *et al.*, 2010).

Fig. 1d. Ripe-brown *T. tetraptera* pod.

Conclusion

The results obtained in this study showed that advancing maturity had effect on both the macro- and micro-nutrients levels of *T. tetraptera* seed and pod. However, the predominance of fat, protein and most of the minerals analyzed in the seed over the pod at both stages of maturity studied, confers the seed with more potential to nourish the body when ingested either as a spice or as a medicinal plant for curative purposes in certain ailments.

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