Journal of Food Security, 2022, Vol. 10, No. 2, 44-52
Available online at http://pubs.sciepub.com/jfs/10/2/1
Published by Science and Education Publishing
DOI:10.12691/jfs-10-2-1

Determination of Nutritional Composition and Selected Phytochemical and Anti-nutrient Content of *Vitex payos* (Chocolate Berry), a Neglected and Underutilized Fruit from Two Kenyan Counties

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Received May 22, 2022; Revised June 25, 2022; Accepted July 04, 2022

Abstract Indigenous fruits, which are usually underutilized, play an important role in food and nutrition security especially in developing countries. *Vitex payos* fruit (chocolate berry) is one such example which is currently gaining popularity in arid and semi-arid parts of Kenya. Fruits of *V. payos* were collected from three wards in each of the two Counties (Kitui and Tharaka-Nithi). A composite sample from each County was obtained by mixing the three ward samples in a ratio of 1:1:1. Standard analytical methods were used in the determination of the physicochemical composition of the fruit samples. Apart from crude fat and protein, significant differences ($P<0.05$) were observed in other fruit sample parameters between the two Counties. Potassium and calcium contents were significantly higher ($P<0.05$) in Kitui compared to Tharaka-Nithi County samples, while phosphorous was significantly lower ($P<0.05$) in the latter than in the former County samples. Niacin, pyridoxine and ascorbic acid were significantly higher ($P<0.05$) in Kitui than in Tharaka-Nithi County samples while thiamin was not detected in both County samples. All phytochemicals and anti-nutrients determined were significantly higher ($P<0.05$) in Kitui than in Tharaka-Nithi County samples. The results from this study indicate that *V. payos* is a good source of nutrients whose concentration may be region-dependent. Nutrient concentration of *V. payos* is comparable to most fruits. Its potassium, dietary fiber and total flavonoid content are particularly higher than in most of the common fruits.

Keywords: *anti-nutrients, chocolate berry, indigenous fruits, nutritional content, phytochemicals, Vitex payos*

Cite This Article: Cyrus Karanja, Samuel Imathiu, Ojijo Nelson KO, and William Thari, "Determination of Nutritional Composition and Selected Phytochemical and Anti-nutrient Content of *Vitex payos* (Chocolate Berry), a Neglected and Underutilized Fruit from Two Kenyan Counties." *Journal of Food Security*, vol. 10, no. 2 (2022): 44-52. doi: 10.12691/jfs-10-2-1.

1. Introduction

Indigenous underutilized fruit trees are important contributors of food and nutrition security particularly in developing countries within subtropical and tropical parts of the world. In these regions, a wide variety of indigenous fruit trees have the potential to curb food and nutrition insecurities [1], but they largely remain untapped possibly due to preference by the farmers and consumers for their exotic counterparts which are perceived as 'fashionable' and more profitable [1]. Underutilization of indigenous fruits even in countries where they are natively abundant is also contributed by consumers' lack of knowledge of their nutritional potential [2], particularly in averting micronutrient deficiencies. Fruits are major contributors of micronutrients in our diets, lack of which leads to micronutrient deficiency (hidden hunger) especially among the women and young children who are the most vulnerable [3]. According to Gödecke et al. [4], more than two billion people suffer from hidden hunger due to inadequate intake of micronutrients in diets which can easily be supplied by fruits (and vegetables).

With the increasing human population and adverse weather conditions occasioned by climate change, more people are exposed to hunger and malnutrition due to lack of enough, nutritious and safe food [5]. Currently, over 800 million people are undernourished, thus chronically hungry in the context of calories intake [6]. This problem is made worse in developing countries where majority of the population are small-holder farmers who rely on a few rain-fed staple food crops. In the event rains fail or are inadequate due to unpredictable weather changes owing to climate change, crop failure occurs leading to food insecurity. In order to overcome this challenge, mitigation strategies by governments and stakeholders including but not limited to food crop diversification and adoption of more resilient climate-smart crops/plants such as indigenous fruit trees needs to be prioritized. Indigenous

fruit trees role in addressing hidden hunger and malnutrition has been documented [7] and their food-poverty reduction is recognized. This therefore means that utilization of indigenous fruits can help in the realization of the United Nation's Sustainable Development Goals one, two and three i.e. no poverty, zero hunger, and good health and well-being respectively [8]. According to Akinnifesi et al. [9] there is a huge potential of indigenous fruit trees from various African regions undergoing domestication and integration in crop farms. In fact a huge uptake of *V. payos* as a fruit tree has been witnessed in recent times in many marginalized part of Kenya which has immensely helped in improving livelihoods among the rural poor [10].

Vitex L. genus consist at least 250 species of trees which are adapted to arid and semi-arid environments and thus are climate resilient. Of the several species within the genus, *V. payos*, also known as chocolate berry has the greatest food potential. *Vitex payos*, which is currently undergoing domestication, is a resilient fruit tree adapted to the drylands of Central, Coastal and Eastern parts of Kenya. People in the growing areas usually collect the fruits of *V. payos* for immediate consumption and subsistence sale in local markets during the ripening season that falls between April and July [2]. Although *V. payos* is an important indigenous fruit tree with a high capability for commercialization in Kenya [11], its use as a food source (micro- and micro-nutrients provision in human diet) is under tapped. This underutilization of the fruit may be as a result of it being viewed as a 'poor man's' fruit that is only suitable for consumption during times of food crisis, especially in regions where the fruit tree is native [10]. Hence, there is a need to understand the nutritional composition of this fruit that has been part of the diet for communities where the fruit tree flourishes. This information is vital as it can form the basis for promoting the fruit for utilization as food in Kenya. In addition, the processing and value-addition information of *V. payos* is dearth [2]. Promotion of the utilization of *V. payos* fruit through value-addition will contribute to food and nutrition security and improve the livelihoods of persons who rely on the fruit tree to generate income when there is a staple food crop failure often occasioned by unpredictable weather changes due to climate change. The aim of this study was to determine the nutritional profile as well as selected phytochemical and antinutrient content of *V. payos* fruits originating from two Kenyan Counties.

2. Materials and Methods

2.1. *Vitex payos* Fruit Sample Collection Sites

Vitex payos fruits ripen between the months of April and July in areas where the fruit trees grow in Kenya. Fruit samples were collected in July 2021 from three wards in each of the two regions/Counties (Kitui and Tharaka-Nithi) which represented two agro-ecological zones. These regions were chosen due to their abundance in *V. payos* fruit trees. In Kitui County, physiologically mature fruits were harvested from Kiatine, Tulia and Mumbuni wards while in Tharaka-Nithi, freshly fallen and ripe fruits were handpicked from the ground in Ganga, Mwimbi and Muthambi wards. Approximately 30 kg of fruit samples were collected from each ward. Kitui and Tharaka-Nithi Counties are in agro-ecological zones IV and V with characteristics shown in Table 1. These zones are considered semi-arid regions that often do not receive enough rainfall to support rain-fed agriculture [12].

2.2. Fruit Sample Preparation and Storage

Fruit samples were collected in plastic crates which were appropriately stacked and transported at ambient temperature to the Food Processing Workshop (FPW), Department of Food Science and Technology at Jomo Kenyatta University of Agriculture and Technology. Upon arrival at the FPW, they were sorted out by removing extraneous material and unsound fruits. Fruit samples from Kitui County were stored at room temperature (approximately 24°C) for five days to ripen before they were transferred to the cold store (4°C), while those from Tharaka-Nithi County were directly stored in the cold room.

2.3. Composite Sample Preparation and Pulping

Two composite samples (Kitui and Tharaka-Nithi) were prepared by mixing the three ward samples from each County in a ratio of 1:1:1 i.e. Kiatine, Tulia and Mumbuni ward samples formed a composite Kitui sample while Ganga, Mwimbi and Muthambi ward samples formed Tharaka-Nithi composite sample. These were then pulped using a pulper finisher-juice extractor (HCR No. 0402, Hashimoto Canning Research Institute, Tokyo, Japan) to separate the seed from the flesh. Seeds were discarded while the pulp was packaged in 250g food grade plastic containers and frozen at -20°C until they were required for analysis. All determinations were carried out in triplicates.

2.4. Determination of Proximate Composition of *Vitex payos* Fruit Pulp

AOAC [15] methods were used to determine moisture, crude protein, ash, crude fat and crude fiber.

2.4.1. Determination of Moisture Content

Two grams (initial weight, W_1) of fruit pulp samples were weighed into a moisture dish and put into an oven which had been preheated to temperatures of 105°C after which the sample was dried for 1 h. The dried sample was then cooled in a desiccator and its final weight (W_2) taken. Percentage moisture content was determined as follows;

$$\%Moisture = \left[(W_1 - W_2) / W_1 \right] x100$$

Where;
W_1 = Sample weight before drying
W_2 = Sample weight after drying.

Table 1. Characteristics of agro-ecological zones IV and V in respect to annual mean temperatures and rainfall

County	Agro-ecological zone	Annual mean temperatures (°C)	Annual mean rainfall (mm)	Altitude (meters above sea levels)	Soil type
Kitui	IV	20.9-24.0	720-1000	600-900	loam
	V	23.0-24.0	550-790	600-900	loam
Tharaka-Nithi	IV	21.0-23.5	820-920	250-1500	sandy loam
	V	22.9-24.0	600-900	250-1500	sandy loam

Source: Manzi and Gweyi-onyango, 2020 [12]; Onyango and Mathooko, 2013 [13] and MoALF, 2017 [14].

2.4.2. Determination of Crude Protein

A one gram of fruit pulp sample was weighed into a digestion flask containing a catalyst made of 0.5g of CuSO4, 15 mL of concentrated H_2SO_4 and 5g of K_2SO4. The content was heated in a fume hood until the mixture turned blue indicating the end of the digestion process. The content was then cooled and transferred into a flask which was then topped up to the 100 mL mark using distilled water. A control with only the catalysts and the acid was also prepared. Ten millilitres of diluted digest was transferred into a flask after which it was washed using 2 mL of distilled water. This was followed by addition of 15 mL of 40% NaOH and washing with 2 mL distilled water. The distillation was carried out on 60 mL distillate followed by titration using 0.02N HCl, an orange colour signified the end point [15]. The protein content was calculated using the formula;

$$\%\ Crude\ protein = (V_1 - V_2) \times N \times f \times 0.014 \times 100 / V \times 100 / S)$$

Where;
V_1 = Sample titer (mL)
V_2= Blank titer (mL)
N = Normality of standard 0.02N HCl solution
V = Volume of diluted digest taken for distillation (10 mL)
S= Sample weight (g)
f = 6.25 (protein factor)

2.4.3. Determination of Ash Content

Ash content of respective pulped fruit samples was determined by dry ashing method according to AOAC [15] method. Five grams of a sample was weighed into a crucible of a known weight. The sample was then charred in a fume chamber after which it was transferred into a muffle furnace and the temperature gently elevated to 550°C. The samples were then ashed for roughly 5 h after which the temperature was lowered gradually to 100°C. The samples were then transferred into desiccators for cooling. Final weight of the crucible and ash was then taken and ash content calculated using the formula;

$$\%Ash = \left[\begin{pmatrix} weight\ of\ crucible\ and\ ash \\ -\ weight\ of\ empty\ crucible \end{pmatrix} / \ sample\ weight \right] x100$$

2.4.4. Determination of Crude Fiber Content

A two gram of a pulped fruit sample was weighed and put into 500 mL conical flask and then 200 mL of boiling 1.25% H_2SO_4 was added and boiled for 30 min under a reflux condenser. The mixture was then filtered under a mild vacuum with a Pyrex glass filter and the residue rinsed to completely remove the acid using boiling water. A 200 mL of boiling 1.25% NaOH was added into the washed residue and boiling done under reflux for another 30 min. The glass filter that had been previously used with acid was again used to filter the residue. Using boiling water, the residue was rinsed followed by 1% HCl. It was then washed using boiling water to remove the acid. Using 99.8% alcohol, the residue was cleansed twice followed by cleansing three times using ether. The residue was then oven dried at 105°C to a constant weight followed by incineration in a muffle furnace at 550°C for 3 h. The content was then cooled in a desiccator and the final weight taken.

Crude fibre content was computed using the formula below;

$$Crude\ fibre\ (\%) = \left[(W_1 - W_2) / W \right] x\ 100$$

Where;
W_1 = Acid and alkali digested sample weight
W_2 = Incinerated sample weight after acid and alkali digestion
W = Sample weight

2.4.5. Determination of Carbohydrates Content

Carbohydrates concentration was determined using anthrone reagent method [16] where carbohydrates are first hydrolyzed into simple sugars (expressed as glucose) using percholic acid. Glucose is then dehydrated in hot acid medium to hydroxymethly furfural, a compound that forms a green colored product with anthrone reagent with a maximum absorption at 630nm. Pulped fruit sample portions of 0.5 g were weighed into 50 mL conical flask and homogenized in hot 80% ethanol to remove sugars. The residue was then centrifuged and retained. This residue was repeatedly washed with hot 80% ethanol until the washing stopped giving colour with anthrone reagent. The residue was then dried over a boiling water bath (100°C). To this residue, 5.0 mL of water and 6.5 mL of 52% of percholic acid was added. The residue was then extracted at 0°C for 20 min. The mixture was then centrifuged and the supernatant saved. Extraction was repeated on the residue using fresh percholic acid, then the mixture was centrifuged and the supernatant pooled together with the earlier obtained supernatant and the volume made up to 100 mL. From the supernatant, 0.2 mL was pipetted and the volume made up to 1 mL with water. A standard was prepared by taking 0.3, 0.4, 0.6, 0.8 and 1 mL into separate tubes with water after which 4 mL of anthrone reagent was added to each tube. The tubes were then heated in a boiling water bath for 8 min. The tubes were then cooled rapidly and the intensity of green colour to dark green colour read using a colorimeter at 630 nm. Carbohydrate content was determined using a standard graph.

2.4.6. Determination of Crude Fat Content

Crude fat content was determined using Soxhlet's extraction method where a 5 g of fruit pulp sample was weighed into a Soxhlet thimble. The thimble top was closed with a defatted cotton wool, weighed and the weight recorded. The thimble was then inserted in the Soxhlet's apparatus and extraction was done for 2 h using petroleum spirit at 40-60°C in a reweighed round-bottomed flask (the round-bottomed flask had been weighed with anti-bumping granules before extraction). After extraction, the solvent was distilled off from the round-bottomed flask using the rotary evaporator. The round-bottomed flask was then dried off in the oven and then weighed with the extracted oil. Percentage crude fat content was calculated using the formula;

$$\%Fat\ content = \left[\begin{pmatrix} weight\ of\ flask\ with\ oil \\ -weight\ of\ empty\ flask \end{pmatrix} / weight\ of\ sample\right] x100$$

2.5. Determination of Mineral Content

Mineral content was determined by dry ashing and atomic absorption spectrophotometry according to AOAC [15] method. Five grams of pulped fruit sample was weighed into a crucible, which was then charred on a hot plate under a fume chamber by gently raising the temperature. Charred samples were then transferred into a muffle furnace and temperature increased to 250°C and held for 1 h. The temperature was then increased to 600°C and samples incinerated for 5 h. The temperature was then reduced to 300°C after which the crucibles were transferred into a desiccator and cooled to room temperature (24°C). Twenty millilitres of 0.5 HNO_3 was added into the ash and the mixture transferred quantitatively into a 100 mL beaker and heated at 80-90°C on a hot plate for 5 min. This was then transferred to a 100 mL volumetric flask and filled to the mark using 0.5N HNO_3. A 10 mM of lanthanum chloride was added to achieve 0.5% in the sample solution for calcium determination. Insoluble matter was filtered and the absorbance of the solution determined by atomic absorption spectrophotometer (Shimadzu AA-7000). Various mineral standards were prepared to make respective minerals calibration curves, which were then used to determine the concentration of specific minerals.

2.6. Determination of Water-Soluble Vitamins Content

Water-soluble vitamins were determined using the method described by Ekinci and Kadakal [17]. Twenty milliliters of deionized water was added into 5 g of the sample. The mixture was then homogenized using a homogenizer (Polytron, Model PCU11, Kinematic AG Litau, Switzerland) at medium speed for a minute. Homogenized samples were centrifuged at 14 x $10^3 g$ for 10 min. Samples were handled using solid phase extraction with sep-pak C18 (500mg) cartridges that enabled separation of water-soluble vitamins and removed most of the meddling constituents. The stationary phase was then flushed with 10 mL methanol and 10 mL water, and adjusted to a pH of 4.2 to make the stationary phase active. Centrifuged samples (10 mL) were then loaded in flushed cartridges (referred above). The samples were eluted with 5 mL water (pH 4.2) followed by 10 mL methanol at a flow rate of 1 mL/min. The eluent was collected in a 50 mL spear shaped flask and evaporated at 40°C in a rotary vacuum evaporator after which the residue was dissolved in the mobile phase. All the samples were filtered through 0.45 µm pore size filter HPLC analysis. A 20µL of the filtrate (solutions of the water-soluble vitamins) were injected into the HPLC connected with photo-diode detector (Shimadzu SPD20A series) at 265 nm for ascorbic acid, 234 nm for thiamine, 234 nm for pyridoxine, and 261 nm for niacin. The mobile phase was filtered through a 0.45 µm membrane and degassed by sonication before use. The mobile phase used was 0.1mol/l KH_2PO_4 (pH 3.6) A and B as 70% acetonitrile mixed with 30% solvent A and reverse phase column (ODS C18, 250mm x 4.6mm x0.5uL). The flow rate was at 1.0 mL/min at 25°C. The water-soluble vitamins identification and quantification was achieved by comparing their retention times with those of standards of known concentrations.

2.7.1. Determination of Total Polyphenol Content

Total polyphenol content was determined using the adjusted Waterman and Mol [18] method where 0.25g of a sample was weighed and put in an amber glass bottle. Fifty millilitres of methanol was added and secured properly and the extraction carried out for 3 h in a shaker (250 motions per minute). The extract was then held in the dark for 72 h for further extraction and then filtered using the Whatman No. 1 filter paper. The extract was topped up to 50 mL using methanol which was then centrifuged for 10 min at 25°C at 150 rpm. One milliliter of the supernatant was filtered through 0.45 µm micro-filter and put in a test tube. Two milliliters of 10% Folin Ciocalteu reagent was added into the test tube and the mixture vortexed for 2 min after which 4 mL of 0.7 M Na_2CO_3 was added and vortexed again for 2 min. The mixture was then left for 2 h to develop color. The readings were made using UV-VIS spectrophotometer (Shimadzu UV-VIS 1800) at 765nm with software UV-VIS probe.

2.7.2. Determination of Total Flavonoids Content

Total flavonoid content was determined using aluminum chloride colorimetric method as described by Jagadish et al. [19] where 0.25g of a sample was weighed and put in an amber glass bottle. Fifty millilitres of methanol was added and secured properly and the extraction carried out for 3 h in a shaker (250 motions per minute). The extract was then held in the dark for 72 h for further extraction and then filtered using the Whatman No. 1 filter paper. Four millilitres of distilled water was put into a 10 mL test tube and 1 mL of the extract added to it and held for 3 min. Into this mixture, 0.3 mL of 5 % $NaNO_2$ solution was added. After settling for 3 min, 0.3 mL of 10 % $AlCl_3$ was added and again allowed to settle for a further 5 min. Two milliliters of 1M NaOH was added and distilled water used to make the volume up

to 10 mL. A UV-Vis spectrophotometer (Shimadzu model UV –VIS 1800, Kyoto, Japan) was used to take the absorbance readings at 415 nm. A calibration curve of standard prepared from quercetin was used to determine the amount of total flavonoids.

2.8. Determination of Anti-nutrients Content

2.8.1. Determination of Oxalates Content

Analysis for oxalates was carried out using the HPLC method detailed by de Guevara et al. [20] with modifications recommended by Yu et al. [21]. Five grams of pulped fruit sample was homogenized in 4 mL of 0.5N HCL. The homogenate was then heated at 80°C for 10 min with intermittent shaking. Distilled water was then added to the homogenate to make a volume of 25 mL. Three milliliters of the solution was withdrawn and centrifuged for 10 min at 12000 rpm after which 1 mL of supernatant was passed through a 0.45µm micro-filter and then analyzed by HPLC using Shimadzu UV-VIS detector. Standards were prepared at different concentrations for quantification. The solid phase was Hypsil C18 column (250 mm x4.6 m x5 µl) while the mobile phase was a solution of 0.01 N H_2SO_4. The flow rate was at 0.6 mL min^{-1} under the pressure of 11.5 kpa and detection was set at a wavelength of 221 nm.

2.8.2. Determination of Phytates Content

Phytate content was determined using the methodology described by Camire [22]. Ten milliliters of 3% H_2SO_4 was used to extract 0.5 g of the sample. The content was then filtered into 50 mL centrifuge tubes and the filtrate placed into a boiling water bath for 5 min followed by addition of 3 mL of $FeCl_3$ solution (6 mg ferric iron per mL in 3% H_2SO_4). To complete precipitation of the ferric phytate complex, the content was heated for 45 min followed by centrifugation for 10 min at 2500 rpm after which the supernatant was discarded. The precipitate was washed using 30 mL of distilled water, centrifuged and the supernatant discarded. To the residue, 3 mL of 1.5 N NaOH was added and the volume made up to 30 mL using distilled water. This was followed by heating of the content in a boiling water bath for 30 min to precipitate the ferric hydroxide. Samples were then cooled, centrifuged, and the supernatant transferred into a 50 mL volumetric flask. This was followed by rinsing the precipitate with 10 mL of distilled water, centrifuging and adding the supernatant to the content of the volumetric flask. Using 0.45µm membrane, the content was micro-filtered in readiness for analysis using the HPLC which was carried out using Shimadzu Refractive Index Detector (RID- 6A). The composition of mobile phase was 0.005 N $C_2H_2NaO_2$ (sodium acetate) in distilled water, at a flow rate of 0.5 mL/min through a reverse phase column (ODS C18, 250mm x 4.6mm x0.5ul).

2.9. Statistical Analysis

Data was subjected to one-way ANOVA using StataSE 12 (64-bit) software. Results were expressed as mean ± standard deviation. Significant difference was determined at $P<0.05$.

3. Results and Discussion

3.1. Proximate Composition

A comparison of nutritional composition of *V. payos* fruits from Kitui and Tharaka-Nithi Counties is presented in Table 2. *Vitex payos* fruit samples from both regions were found to contain substantial amounts of protein, fiber, fat, carbohydrates and ash. Apart from crude protein and crude fat contents, significant differences ($P<0.05$) in other parameters (moisture, crude fibre, carbohydrates and ash) were observed. *Vitex payos* fruit samples from Kitui County were significantly higher in moisture content, fiber and ash contents compared to those from Tharaka-Nithi County. Various factors can affect and influence the concentrations nutrients of a fruit variety grown under different climatic conditions. It is therefore not surprising that certain parameters differed significantly for *V. payos* from the two regions. The soil characteristics, altitude and weather conditions among other factors are known to influence crop quality [23].

Significantly higher levels in ash in Kitui County samples may be because soils in this region were richer in minerals compared to those from Tharaka-Nithi County. Results from this study indicates that *V. payos* fruit is superior in ash content compared to popular fruits such as apples, pears, grapes, strawberries, oranges and ripe bananas, among others [24] and thus can immensely contribute in mitigating 'hidden hunger' resulting from micronutrients deficiency. On average, it was observed that *V. payos* fruit pulp contained about 70% moisture content, with Kitui County samples containing significantly higher amount (72.631±2.85%) than Tharaka-Nithi County samples (67.801±0.45%). Fruit moisture content can be influenced by the ability or lack of it of the soil to retain moisture which to some extent is dependent on soil's organic matter content and the type of soil. Table 1 shows that Kitui County soils are predominantly loam while Tharaka-Nithi County soils are generally sandy loam. Loam soils generally retain more moisture [25] and therefore, it is not surprising that Kitui samples on average had higher moisture content than the Tharaka-Nithi County samples. Kimondo et al. [2], in a study investigating physicochemical and nutritional characterization of *V. payos* found similar results in regard to the fruit's moisture content. The authors reported a mean moisture content of 68.4 ±0.4% which is comparable to findings in this study. With a fiber content of 42.21±4.62 g/100g for Tharaka-Nithi County and 55.59±5.50 g/100g for Kitui County, *V. payos* fruit is rich in this important dietary component compared to most of the fruits, findings that are in agreement with those reported by Kimondo et al. [2] on the same fruit species. Sufficient dietary fiber intake is scientifically linked to reduced incidences of obesity and cardiovascular illnesses [3] and *V. payos* fruit being rich in this, can be promoted to fight these non-communicable diseases.

Table 2. Nutritional composition of *V. payos* fruits from Kitui and Tharaka-Nithi Counties. Values (g/100g-dwb) are means of three replicates. Means with different superscripts along the same column are significantly different from each other (p<0.05)

Region/County	Parameter						
	Moisture content	Protein content	Fiber content	Fat content	Carbohydrate content	Ash content	Energy* (kj/100g dwb)
Kitui	72.631±2.85^{b}	2.25±0.16^{a}	55.59±5.50^{b}	1.86±0.09^{a}	28.27±4.43^{a}	6.10±0.37^{b}	587.60±81.20^{a}
Tharaka-Nithi	67.801±0.45^{a}	2.43±0.11^{a}	42.21±4.62^{a}	2.20±0.31^{a}	48.33±4.48^{b}	4.83±0.11^{a}	944.30±76.10^{b}
P-value	0.044	0.168	0.032	0.148	0.005	0.005	0.005

On the contrary, the samples from Tharaka-Nithi County were significantly higher (P<0.05) in carbohydrates and energy content than those from Kitui County. The carbohydrates content of Tharaka-Nithi County samples was almost double those of Kitui County samples (48.33±4.48 g/100g and 28.27±4.43 g/100g respectively), an observation that was also reflected in energy contents of the respective samples, 944.30±76.10 kj/100g for Tharaka-Nithi County and 587.60±81.20 kj/100g for Kitui County. This observation suggests a direct relationship between the level of carbohydrates and energy content and an indirect relationship between the amount of fibre and the energy content (Table 2). Upon a subjective fruit samples taste test, Tharaka-Nithi County samples were found to be sweeter than those from Kitui County. These differences may be due to varying environmental conditions under which the fruit trees grew in the respective study regions. Just like in the majority of most fruits, *V. payos* is not an excellent source protein and fat.

3.2. Mineral Composition

Table 3 presents results of mineral content of *V. payos* fruits from the two regions under the study. Although the daily quantities of mineral nutrients required by human body is minimal compared to macronutrients, they are vital for proper body functions, without which growth and/or health is compromised [26]. Both the macro- and micronutrients determined in this study occurred in levels that could adequately contribute to human dietary requirement (Table 3). There were no significant differences (P>0.05) in the levels of sodium, magnesium, zinc and iron between Kitui and Tharaka-Nithi County samples. Significant differences (P<0.05) were however observed in the levels of phosphorous, potassium and calcium between samples from the two Counties with those from Tharaka-Nithi County containing significantly higher phosphorous than that from Kitui County (48.44±4.44 and 31.54±4.99 g/100g respectively). Samples from Kitui County contained significantly higher potassium compared to those from Tharaka-Nithi County (2331.60 and 1816.60 g/100g dwb respectively). Likewise, the calcium content of Kitui County samples was statistically higher (P<0.05) than those from Tharaka-Nithi County (75.09±13 and 49.10±11 g/100g dwb respectively).

Differences in the concentration of minerals between samples from the two Counties may have been influenced by the differences in regional soil composition in terms of inherent soil mineral content which is likely to influence the levels in the fruits. Trace minerals (zinc and iron) occurred in lowest levels ranging from 2 to 3 mg/100g compared to the micronutrients. These levels are however, significantly higher than those reported for most of the fruits [24]. Macrominerals levels determined in this study were higher than those reported by Kimondo et al. [2] in a similar study. This difference may be attributed to among other factors; type of the soils on which the fruit trees grew, stage of fruits harvesting and the analytical techniques used in the quantification. On average, of all minerals, potassium occurred in the greatest concentration while zinc occurred in the lowest concentration. This study has revealed and is in agreement with findings of Kimondo et al. [2] that *V. payos* is way superior in potassium concentration than most of the documented fruits. In fact *V. payos* potassium content is higher than that of another potassium-rich neglected underutilized indigenous tropical fruit, *Adansonia digitata* which has been reported to contain 1793.8±30mg/100g-dwb [27].

Table 3. Mineral content of *V. payos* fruits from Kitui and Tharaka-Nithi Counties. Values (mg/100g-dwb) are means of three replicates. Means with different superscripts along the same column are significantly different from each other (p<0.05)

Region/County	Mineral						
	Phosphorous	Sodium	Potassium	Magnesium	Calcium	Zinc	Iron
Kitui	31.54±4.99^{a}	46.41±9.15^{a}	2331.60±185.70^{b}	69.06±12.98^{a}	75.09±13.39^{b}	2.00±0.15^{a}	2.67±0.34^{a}
Tharaka-Nithi	48.44±4.44^{b}	38.24±9.39^{a}	1816.60±222.20^{a}	55.14±4.93^{a}	49.10±11.03^{a}	2.10±0.18^{a}	2.61±0.30^{a}
P-value	0.012	0.341	0.037	0.158	0.040	0.483	0.831

Table 4. Water soluble vitamin concentration of *V. payos* fruits from Kitui and Tharaka-Nithi Counties. Values (mg/100g-dwb) are means of three replicates. Means with different superscripts along the same column are significantly different from each other (p<0.05)

Region/County	Vitamin			
	Niacin (Vitamin B3)	Pyridoxine (Vitamin B6)	Thiamine (Vitamin B1)	Ascorbic acid (Vitamin C)
Kitui	18.90±1.52^{b}	6.81±0.98^{b}	n.d	8.56±0.98^{b}
Tharaka-Nithi	6.27±0.31^{a}	4.36±0.05^{a}	n.d	2.70±0.05^{a}
P-value	0.000	0.010		0.001

n.d.: not detected.

3.3. Water Soluble Vitamins

Foods rich in fruits (and vegetables) are highly recommended in human diets by medical health and human nutrition practitioners worldwide because of their health-promoting potential [3]. Fruits are particularly great sources of a wide range of essential vitamins required for various body functions. All the vitamins determined were detectable and quantifiable except thiamine (vitamin B1) as shown in Table 4. Niacin (vitamin B3), pyridoxine (vitamin B6) and ascorbic acid (vitamin C) were statistically higher (P<0.05) in Kitui County than in Tharaka-Nithi County samples. Of the quantifiable vitamins, niacin occurred in the highest concentration (6.27±0.31 mg/100g and 18.90±1.52 mg/100g for Tharaka-Nithi and Kitui Counties respectively).

Vitex payos fruit samples from Kitui County were three time higher in niacin and ascorbic acid, and almost two times higher in pyridoxine compared to Tharaka-Nithi County samples. The reason for statistically higher concentrations of the quantified vitamins from Kitui County samples than those from Tharaka-Nithi County is not clear, but could be due to differences in the environmental conditions in which the fruit trees grew or may also have been influenced by the stage at which samples were collected. Kitui County samples were harvested when they were physiologically mature and ripened for five days while ripe samples were collected from Tharaka-Nithi County. Findings of this study contradicts those of Palgrave [28] who reported that *V. payos* fruits do not contain vitamin C. On the other hand, Kimondo et al. [2] observation differs from this study's findings in that these authors reported significantly higher vitamin C (26.3±4.9mg/100g) and lower niacin (0.58±0.2 mg/100g) and pyridoxine (0.11±0.032 mg/100g). Compared to tropical fruits such as lime (46·5 mg/100g), grapefruit (47·0mg/100g) and pawpaw (43.2 mg/100g) [29], *V. payos* fruit is an inferior source of vitamin C. The fruit is however higher in pyridoxine content compared to oranges, apples and strawberries among other others [24].

3.4. Total Polyphenol, Total Flavonoids and Anti-Nutrients Content

Table 5 presents results for total phenolics, total flavonoids, phytates and oxalates quantified from *V. payos* fruits from the two regions under the study. In addition to being good sources of vitamins and minerals, fruits (and vegetables) are also dietary sources of phytochemicals that have protective capabilities including acting as antioxidants and anti-inflammatory agents [3]. Phytochemicals are bioactive compounds, usually considered non-nutrients that aid in scavenging toxic radicals through the production of antioxidants, thus contributing in the mitigation of many chronic illnesses [30]. For all the parameters determined, statically higher concentrations (P<0.05) were observed in Kitui County samples than in Tharaka-Nithi County samples. Total flavonoids and phytates were 17.6 and 3.6 times higher in Kitui County samples than in Tharaka-Nithi County samples respectively (Table 5).

Vitex payos fruit sample from Kitui and Tharaka Nithi Counties had total phenolic contents of 270.75±31.12 mg gallic acid equivalent (GAE) $100g^{-1}$ and 150.8±1.72 mg GAE $100g^{-1}$ respectively, and total flavonoids of 7977±913.12 mg quercitine equivalent(QE) $100g^{-1}$ and 452±3.4 mg QE $100g^{-1}$ respectively. The total phenolic contents of the fruit pulps in both cases were lower but close to that of *V. doniana* collected from three regions in Cote d'Ivoire which ranged from 202.51±4.19 to 463.65±6.85 mg GAE $100g^{-1}$. On the other hand, total flavonoid content of *V. payos* from both Kitui and Tharaka Nithi Counties were much higher than that of *Vitex doniana* collected from three regions in Cote d'Ivoire which ranged from 75.71±1.03 to 145.55mg QE $100g^{-1}$ [31]. The difference in total phenolic and flavonoids values between the fruit samples from the two Counties could be attributed to the difference in geographical locality while the difference between the values and that from literature could be associated to both the difference between the countries of study (locality) and the species of the fruits involved [31].

Antinutrients are substances, which reduce the nutrients utilization and /or food intake in plant or plant-based products used as human food. The quantity and distribution of anti-nutrients varies with plant genera and species. Some of the antinutrients commonly found in leafy vegetables and fruits are nitrates, oxalates, tannins, phytates and cyanogenic glycosides [32]. *Vitex payos* pulp from Kitui and Tharaka Nithi Counties had higher content of phytates (189.48±14.44mg $100g^{-1}$ and 53.15±13.64mg $100g^{-1}$ respectively) and lower content of oxalates (126.75±6.67mg$100g^{-1}$ and 106.32±0.97mg $100g^{-1}$ respectively) compared to *V. doniana*. The latter was reported to have a phytate content of 75±0.16mg $100g^{-1}$ and oxalate content of 1000.1mg±2.12mg $100g^{-1}$ [33]. This implies that most beneficial phytochemicals and minerals may be chelated by these antinutrients when *V. payos* fruit is consumed. However, the level of oxalic and phytic acids may be effectively reduced by blanching of the fruits [32], an investigation worth recommending for future study.

Table 5. Selected phytochemicals and anti-nutrients composition of *Vitex payos* fruits from Kitui and Tharaka-Nithi Counties. Values are means of three replicates. Means with different superscripts along the same column are significantly different from each other (p<0.05)

Region/County	Parameter			
	Total phenolics (mg GAE$100g^{-1}$, dwb)	Total flavonoids (mg QE $100g^{-1}$,dwb)	Phytates mg/100g-dwb	Oxalates mg/100g-dwb
Kitui	270.75±31.12^{b}	7977.1±913.00^{b}	189.48±15.44^{b}	124.75±6.67^{b}
Tharaka-Nithi	150.88±1.74^{a}	452.2±3.40^{a}	53.15±13.64^{a}	106.32±0.97^{a}
P-value	0.003	0.001	0.001	0.009

4. Conclusion

This study has shown that *V. payos* fruit is a good source of nutrients, the levels of which are comparable to most commonly consumed conventional fruits and in some instances exceeds their concentration. Of particular interest is the concentration of potassium, dietary fiber and total flavonoids in this fruit, which was significantly higher than the concentrations reported for most fruits. Based on this, it is possible to promote the consumption of this fruit as a probable health-promoting food across different classes of people and help shedding off its 'poor man's fruit' tag. The fact that the fruit tree requires little or no inputs to grow in marginal areas of the tropics where most staple food crops do not do well, can go a long way in mitigating hunger, improving nutrition and uplifting livelihoods in these regions. It is however important to note that variability in nutrient, phytochemical and anti-nutrient concentration in *V. payos* fruit may occur due to differences attributable to the fruit tree growing environment. More research needs to be carried out to investigate nutrients bioavailability in relation to anti-nutrient content, and the potential for the production of *V. payos* value-added food products to promote the fruit utilization.

Acknowledgements

Authors would like to acknowledge Mr. David Abuga of the Food Science and Technology Laboratories at Jomo Kenyatta University of Agriculture and Technology for his assistance during sample analysis.

Funding

This research was funded by Africa-ai-Japan Project Innovation Research Fund (2021/22).

Conflict of Interest

Authors declare no conflict of interest.

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