

## Nutritional Potential of *Myrianthus holstii* Fruit of Rwanda

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### Abstract

*Myrianthus holstii* fruit has been a neglected edible fruit with limited nutritional information. This study evaluated the physical and chemical characteristics of *M. holstii* fruit of Rwanda. The physical parameters including fruit weight, fruit size, pulp content, seed weight and seed size are reported. The fruit pulp that is acidic pH ( $3.38 \pm 0.04$ ) contained; protein ( $8.03 \pm 0.95$  g/100g), dietary fibre ( $25.64 \pm 0.06$  g/100g), vitamin C ( $19.80 \pm 2.13$  mg/100g), beta carotene (0.99 mg/100g), iron ( $16.262 \pm 0.576$  mg/100g), zinc ( $2.327 \pm 0.034$  mg/100g) and copper ( $0.573 \pm 0.011$  mg/100g). Assessment of these nutrient contribution to the Recommended Dietary Allowance (RDA) showed 100g of pulp can meet 42%, 103%, 79%, 233%, 163%, 49% and 143% for children (4-8-year-old); and 18%, 103%, 22%, 133%, 90%, 29%, and 63% for adults (19-50years) respectively. The seeds had an oil yield of  $37.67 \pm 1.53\%$  with omega 6 fatty acid (78.92) being the most dominant. The total unsaturation in the oil was 90.91% with 78.92% poly unsaturated fatty acids. Based on the nutritional information, *M. holstii* can be a good source of beta carotene (vitamin A), iron, zinc and omega 6 fats that are essential in nutrition and health. Therefore, the fruit should be promoted for consumption as a snack and also processed into food products like fruit juice, wine, jelly, jam and vegetable oil to enhance the nutrition, health and income of households.

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**Key words:** *Myrianthus holstii*, fruit, nutritional composition, bioactive, proximate, chemical, physical, Rwanda

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## Introduction

*Myrianthus bolstii* Pal. (Urticaceae) commonly referred to as giant yellow is a dominant medium sized fruit tree in the forested areas of Uganda, Tanzania, Kenya, Democratic Republic of Congo, Mozambique, Zambia, Zimbabwe, Cameroon, Burundi and Rwanda. This fruit tree grows up to 10 m high with a short bole and large branches. Its leaves are mostly alternate and stipulate with unisexual flowers and the fruits are around with about 4 cm in diameter and contains numerous seeds surrounded with edible sweet-sour pulp (Katende *et al.*, 1995). The fruits ripen between June and August. *Myrianthus bolstii* is called by different names in the countries they are growing. For instance, in Tanzania, *M. bolstii* is called Chamba; in South Western Uganda (Mugunga and Omufe) and in Rwanda it is called umwufe.

Although fruits of *M. bolstii* have been edible among humans for centuries, it is also an important food for wild animals including chimpanzees, mountain gorilla, baboons and elephants (Rode *et al.*, 2006, Rothman *et al.*, 2007). In fact, most of the nutritional analysis on *M. bolstii* fruit has focused mainly on the diet of animals (Matsumoto-Oda and

Hayashi, 1997, Rothman *et al.*, 2006; Rode *et al.*, 2006; Kamungu *et al.*, 2015). Even the nutritional analysis of *M. bolstii* fruit showed variations and in some cases the results were aggregated as nutritional values of fruits eaten by wild life (Rothman *et al.*, 2007; Reiner *et al.*, 2014) .

Studies that report the nutritional values of *M. bolstii* fruit as human food are scarce. Yet, the United Nations for Food Agricultural Organization (FAO) describes *M. bolstii* as a high prospects new crop for fruit production (Naluswa, 1993). According to Maundu *et al.*, (1999), *M. bolstii* fruit can save the population during periods of famine and recommended it to be a priority species for domestication in Kenya. In Rwanda, *M. bolstii* was identified as a priority indigenous fruit trees species for fruit production because of preference by both children adults (Bigirimana *et al.*, 2016). To enhance the commercial potential of *M. bolstii*, it is better to provide adequate knowledge of its physical and chemical characteristics. This study has comprehensively evaluated the physical, chemical, nutritional and bioactive components of *M. bolstii* from Rwanda.

fruits from different trees were each packed separately, put in a cool box at 4°C and transported to the laboratory where they were stored at -10°C in closed polythene bag until laboratory analysis were done.

## Materials and Methods

### Study area

The study was conducted in Nyamagabe district in the Southern Province of the Republic of Rwanda. Nyamagabe district is divided into 17 Sectors and has a population of 342,112 people (National Institute of Statistics Rwanda, 2013). The district is found at an elevation of 2237 m above sea level and latitude 2° 24', 29.48" and longitude 29° 28'4.69". Nyamagabe district was chosen because it has the highest abundance of *M. bolstii* trees in Rwanda.

### Fruit sampling and harvesting

The fruits of *M. bolstii* were sampled from two sectors (Musebeya and Uwinkingi) in Nyamagabe District based on their abundance. The abundance was established based on the information generated during reconnaissance visits to the 17 sectors, and also in consultation with the District Government Local Leaders. In each sector, five fruiting trees were then selected using simple random sampling (i.e. 10 mature ripe fruiting trees per district).

### Fruit collection/harvesting

Twelve to fifteen ripe fruit samples were picked randomly from different branches for laboratory analysis. Harvested

### Physical measurements of fruit

At the laboratory, fruits from each tree were defrosted for physical characteristics measurements using methods described by Franquin *et al.* (2005). The weight of each fruit was determined using analytical weighing balance and dimensions i.e. length and breadth with a Vernier caliper. The fruits were then de-pulped to separate the seeds. The extracted mature seeds from each fruit were counted. The weight and size measurements were determined in 50% of mature seeds selected using random sampling technique (Shahbazi and Rahmati, 2013).

### Extraction of fruit pulp

The pulp of each fruit was extracted by cutting the fruit the exo-carp (1 cm from outside) with a stainless-steel knife to obtain the endo-carp that was blended to make a sweet-sour taste juice. The percentage weight of the extracted pulp for each fruit was determined as (weight of pulp/ total weight of fruit) \*100. Because of limited resources to conduct laboratory analysis of pulp of each fruit, extracted pulp of

different fruits were pooled together and blended to make a composite sample for chemical analysis.

#### *Preparation of pulp and seeds*

The freshly extracted pooled pulp was divided into two portions. The first portion (1kg) was used for chemical analysis. The second portion (4kg) was dried at 40-50°C in a vacuum oven for 48 hours before it was crushed into fine powder using an electric grinding machine for nutritional proximate analysis. Similarly, the extracted seeds (3.5 kg) were first dried at 40-50°C for 36 hours. The dry seeds were then de-husked manually using a metallic rod to remove the kernels which were further dried for another 72 hours at the same temperature until their moisture content was less than 12%. The dry kernels were then ground using an electric grinder (Brooks Crompton series 2000), to obtain fine powder.

#### **Chemical Analysis**

The freshly extracted fruit pulp was analyzed for pH and titratable acidity (TA) using standard methods in AOAC (1997). pH was analyzed using a portable pH meter model, Hanner, RI02895 USA while the titratable acidity was determined by mixing 10 gm of the pulp with 60 ml of de-ionized-distilled water with three drops of phenolphthalein indicator and then titrated using 0.1 N NaOH until the pH was 8.1 (by colour changing to pink). The volume of the sodium hydroxide, added to the solution, was multiplied by a correction factor of 0.007 to estimate titratable acidity as percentage of citric acid.

#### **Proximate Analysis of the Pulp**

The proximate parameters of the fruit pulp including moisture, total ash, crude protein, crude fat, dietary fibre, total carbohydrates, and energy were analyzed using standard methods in AOAC, (1997). Vitamin C was determined using 2, 6 dichlorophenolindophenol method (Okullo *et al.*, 2010a) and beta-carotene was determined spectrophotometrically using HPLC (Rodriguez-Maya and Kimura, 2004).

#### **Mineral Composition of the Pulp**

The dry pulp of *M. bolstii* (2.0 g) was digested with nitric acid and used in the determination of minerals like K, Na, Ca, Mg, Fe, Zn and Cu using Atomic Absorption Spectrophotometer model AA-63000 using graphite furnace (Okullo *et al.*, 2010a).

#### **Qualitative phytochemical analysis**

#### **Bioactive Components**

The dry fruit pulp (20g) was extracted with Soxhlet apparatus techniques using 96% ethanol (150ml) for four hours. Phytochemicals including reducing compounds, tannins, saponins and alkaloids were identified directly from the ethanol extract (Ogwang *et al.* 2011; Magadula and Tewtrakul, 2010). However, a portion of ethanol extract (25ml) was hydrolyzed with 10% HCl (15ml) by refluxing for 30 minutes. The refluxed solution was cooled and extracted with diethyl ether (60ml) for identification of phytochemical like anthracenoides, coumarins, steroid glycosides, and flavonoides while the acidic solution was used for identification of anthocyanin pigments or anthocyanoides (Culei, 1982). *Identification of reducing compounds* (Fehling's test): Ethanol extract (1ml) was mixed with Fehling I and II (2 ml each) in a test tube and heated in a water bath. Appearance of red precipitates on the bottom of the test tube indicates presence of reducing compounds. *Identification of saponins* (Frothing test): Ethanol pulp extract (5ml) was shaken vigorously with water (5 ml) in a test tube for 10 minutes. Frothing on top of the liquid which persisted for 10 minutes with column of about 5 cm above liquid level was considered as evidence for presence of saponins. *Identification of tannins* (Ferric chloride test): Ethanol extract (2ml) was diluted with 4 ml of water and three drops of ferric chloride were added. Presence of a blue/black precipitate colour showed presence of tannins

*Identification of alkaloids* (Mayer's reagent test): Ethanol extract (15ml) was dissolved in 10% v/v Hydrochloric acid (10 mL) and 10 % v/v ammonia solution (10ml) was added and shaken. The solution then extracted with ether (15ml). The ether extract was evaporated to dryness and 2% HCl solution (2ml) was added followed by 2-3 drops of Mayer's reagents. Formation of opalescence or a yellowish-white precipitate confirmed the presence of alkaloid salts. *Identification of sterols and triterpenes* (Liebermann-Burchard's test): Ether extract (5ml) was evaporated to dryness in a test tube. Acetic anhydride (1ml) and chloroform (1 ml) were dissolved and sulphuric acid (about 1ml) was added from bottom with the aid of pipette. Presence of a reddish brown ring at interface between the two layers confirmed presence of sterols and triterpenes. *Identification of flavonoides* (Shibata's reaction test): Ether extract (5ml) was concentrated to dryness in a water bath and 2ml of 50% methanol was added and warmed. Magnesium ribbon (1cm long) was then added followed by addition of 2 drops of concentrated HCl. Appearance of yellow or orange colour confirmed presence of flavonoides. *Identification of anthracenoides* (Ammonia solution test). Ether extract (3ml) was mixed with 3ml of

25% ammonia solution in a test tube and shaken vigorously. Appearance of brown to reddish colour in ammonium layer indicated presence of anthracenocides. *Identification of coumarins* (Ammonia solution + UV –Light test): Ether extract (5 mL) was evaporated to dryness and 2ml of hot water was added to dissolve followed by addition of 10% ammonium solution (1 ml). The occurrence of a blue or green fluorescence under UV light (360nm) indicated the presence of coumarins. *Identification of anthocyanocides*: Reddish acidic solution (3ml) remaining extraction with ether was mixed with 10% HCl or 10% Ammonia solution. Formation of a violet colour in acidic medium or green/blue colour in an alkaline medium indicates presence of anthocyanin pigments. *Identification of essential oils*: The fruit pulp powder (5g) was extracted with diethyl ether (50ml) by shaking. Presence of a pleasant odour indicate presence of volatile oils.

### **Quantitative phytochemical analyses**

Quantitative analyses including total phenolic compounds, total flavonoids, total anthocyanins and antioxidant activity were conducted on the fruit pulp.

*Determination of total phenols*: The amount of total phenols in the pulp was used to determine by Ciocalteu reagent method (Singleton and Rossi,1965) and Emaldi *et al.*,2004) In brief, 100 mg of pulp samples was extracted with 10 ml of 50% methanol (5 ml) by manual shaking for 30 minutes. The solution was centrifuged at 300 rpm for 10 minutes and decanted into a test tube. 0.1 ml of the extract was diluted with 0.4 ml distilled water. Diluted Folin–Ciocalteu reagent (0.25 ml) and 20% sodium carbonate (1.25 ml) was added and vortexed. The solution was allowed to stand for 15 minutes and the absorbance was measured at 725 nm using Genesys 10 UV spectrophotometer, model 2G21144001, Thermo Electron Corporation, Cambridge, United Kingdom). Different concentrations of gallic acid (0.1-0.60 mg ml<sup>-1</sup>) or 10, 50, 100, 250 and 500 mg/ml were prepared in methanol for preparation of a standard curve and result were expressed in mg gallic acid equivalents (GAE) g<sup>-1</sup> dried extract (mg GAE/g dry fruit weight).

*Determination of total flavonoids*: Aluminium chloride colorimetric method was used to determine total flavonoids (Seal,2012; Borkatoky *et al.* 2013). In brief, 2.0 ml of the fruit pulp methanol extract was mixed 2% AlCl<sub>3</sub> ethanol solution (2ml) and incubated at room temperature for 1 hour. The absorbance was measured at 420 nm using Genesys 10 UV spectrophotometer, model 2G21144001. A

standard curve of rutin was prepared by dissolving 100 mg of rutin in 100 ml of methanol as stock solution i.e. concentration of 1mg/ml. The stock solution was diluted to concentration ranging from 0, 1.5, 0.3, 0.6 and 1.2 mg/ml with distilled water. The results were then expressed as rutin equivalents (mg rutin equivalents per g of dry fruit pulp).

*Determination of total anthocyanin content*: A spectrophotometric method reported by Moo-Huchin *et al.* (2015) was used to determine total anthocyanins. The pulp (1g) was extracted with 30 ml of 95% ethanol/1.5 M HCl (85:15, v:v) stored at temperature of 4°C. The extracts were centrifuged, decanted and transferred to a 50 ml volumetric flask and the volume completed with 95% ethanol/1.5 M HCl. The absorbance was measured at 535nm using Genesys 10 UV spectrophotometer, model 2G21144001. Total anthocyanin content was determined by applying the Lambert–Beer law, calculated as mg/100 g of Fresh Weight, through the formula:  $A_{535} \text{ dilution factor}/E1\%1\text{cm}$ ; 535 (Francis, 1982)

*Determination of anti-oxidant activity*: DPPH free radical scavenging assay standard method was used to determine antioxidant activity (Simirgiotis & Schmeda-Hirshmann, 2010 and Xu *et al.*, 2010). Pulp methanol extract (20 - 100 µl) were mixed with 3.9 ml of freshly prepared DPPH solution (25 mg L<sup>-1</sup>) in methanol, allowed to incubate for 30 minutes. The absorbance was measured at 517 nm (UV-visible spectrophotometer). The capability to scavenge the DPPH radical was calculated, using the equation:

$$\text{DPPH scavenged (\%)} = \frac{\text{Ac-At}}{\text{Ac}} \times 100$$

Where Ac is the absorbance of the control reaction and At is the absorbance in presence of the sample of the extracts. The antioxidant activity of the extract was expressed as IC<sub>50</sub>.

### **Extraction of the Vegetable Oil from the Seeds**

200 g of seed powder was extracted with hexane (2500 ml) using Soxhlet apparatus at 40-60°C for 6 hours. The hexane extract was concentrated using a rotary evaporator and later dried by heating in a vacuum oven at 50°C for 60 minutes. The percentage of the crude oil was determined gravimetrically as percentage content (AOAC, 1997). The extracted oil samples were stored in the refrigerator at 4°C until laboratory analysis was completed.

### Chemical Analysis of Seed Oil

The physico-chemical characteristics of the extracted seed oil of *M. bolstii* including; colour, refractive index, and acid value were determined using standard methods in AOAC (1997).

*Determination of colour:* The oil sample (10ml) was placed in a cuvet and analysed using a lovi bond –Tintometer (model E, S. No. 5064E, United Kingdom. The colours of red, yellow and blue units were adjusted until a perfect colour match was obtained. The value of the colour with the lowest unit was subtracted from the colours leaving two units which were then used to describe the colour of the sample..

*Determination of refractive index:* The oil samples was analysed directly using Bellingham + Stanley refractometer (Model No. A86006) (AOAC, 1997, United Kingdom.

*Determination of acid value:* The oil sample (2g) was dissolved in neutral diethyl ether (25ml), ethanol (25ml) and 1% phenolphthalein solution (1ml). The solution was titrated with 0.1M NaOH until a pink colour that persisted for at least 15 second was obtained.

$$\text{Acid value} = \frac{\text{Titration (ml)} \times 5.61}{\text{Weight of sample}}$$

*Fatty acid profile:* The fatty acid profile of the oil as methyl esters was determined with a GC-MS (Okullo et al., 2010b).The oil sample (10.0 mg) was mixed with 1ml anhydrous methanol containing 2M HCl and heated in an oven for 2 hours at 90°C. The methanol was evaporated down to about 0.5 ml by a stream of nitrogen gas and 0.5ml

### Results and discussion

#### Physical characteristics

The results for physical parameters of *M. bolstii* of Rwanda is presented in table 1. Although a significant different ( $P \leq 0.05$ ) was observed for fruit weight, seed weight, seed breath; fruit length, fruit width, pulp content. number of seeds and seed diameter of *M. bolstii* fruit collected from of ranged from Uwinkingi and Musebeya Sector, Nyamagabe District, Rwanda .

distilled water added followed by hexane (1ml). The solution was centrifuged and the hexane layer was pipetted and transferred to the vial. One microlitre of hexane layer was injected splitless into GC-MS (An Agilent 6890 N , USA) with column (CP-WAX 52CB Chrompack 25 m x 0.25 mm (1.d.) with hydrogen as mobile phase. Injector and detector temperature were set at 260°C and 330°C respectively. The oven temperature was programmed as 90°C for 4 minutes and electron impact (EI) ionisation was at 70 eV . The ion source temperature was at 230°C. The components eluting from the column as chromatographic peaks were identified by comparison with the standard chromatograph of the mixture of 20 fatty acids methyl esters, GLC reference standard 68D Nu-Check –Prep (Elysian , Minn., USA).. The amount of each fatty acid in the sample was expressed as percent of the sum of all fatty acids in the sample.

#### Data analysis

The data for physical characteristics was summarized using descriptive statistics of range, mean and standard deviation. Student t -test was used compare the physical parameters and Pearson's correlation was used to obtain relationship between these physical characteristics. The data for chemical characteristics were summarized as mean and standard deviation. The nutritional contribution per nutrient was computed as a percent of the Recommended dietary allowance (RDA) standards for children (4-8 years) and female adults (19-50 years) (National Academy of Science, 1989). The RDA value of *M. bolstii* were compared with that of mango pulp (Maldonado-Celis et al., 2019)



Physical parameter	Uwinkingi Sector		Musebeya Sector	
	Mean	Range	Mean	Range
Fruit weight (g)	218.5±84.7 <sup>a</sup>	116.4-408.3	269.1±75.8 <sup>b</sup>	129.5-403.4
Fruit width (cm)	6.74±1.12 <sup>a</sup>	4.96-8.68	7.39±1.19 <sup>a</sup>	4.30-9.40
Fruit length (cm)	6.91±1.13 <sup>a</sup>	4.93-9.32	7.55±0.94 <sup>a</sup>	5.40-9.12
Ratio of Length: Width (shape)	1.02		1.03	
Pulp content (%)	87.55±4.43 <sup>a</sup>	81.26-95.76	84.27±4.81 <sup>a</sup>	73.29-91.51
No of mature seeds	8.5±5.6 <sup>a</sup>	1.0-22.0	11.3±5.0 <sup>a</sup>	4.0-22.0
Seed weight	1.80±0.67 <sup>a</sup>	0.82-2.88	2.83±0.55 <sup>b</sup>	1.51-4.25
Seed diameter (cm)	1.50±0.17 <sup>a</sup>	1.21-1.90	1.55±0.11 <sup>a</sup>	1.30-1.77
Seed breadth (cm)	1.22±0.17 <sup>a</sup>	1.04-1.62	1.12±0.10 <sup>b</sup>	0.91-1.30
Ratio of Diameter: breadth (shape)	1.23		1.38	

Table 1: Physical parameters of *M. holstii* fruit of Uwinkingi and Musebeya Sectors of

The ratio of fruit length to fruit width and seed diameter and seed breadth of *M. holstii* indicate round shaped fruits and oval shaped seeds. Knowledge of weight and size of fruits to farmers, processors, plant breeders and traders is essential for selection of propagation seed, processing technologies, breeding purposes and pricing respectively (Shahbazi and Rahmati, 2013). In marketing, consumers physically tend to prefer fruits that are big in size with equal weight and uniform shape (Demirsoy and Demirsoy, 2004; Usenik *et al.*, 2005). Based on current size and weight measurements, fruit of *M. holstii* can be described as heavy fruits that can attract the attention of consumers and can also fetch high market prices. Nonetheless, high pulp content (84-87%) of *M. holstii* fruit can be essential to consumers for processing products like juice, wine, jelly and jam. Assessment of relationship of the physical parameters is important for plant breeders. In this study, a strong positive correlation ( $R^2=0.833$ ) was observed for fruit weight and fruit width, implying that with increase in width of the fruit the weight automatically increases.

## Nyamagabe district

Phenotypic characteristic	Fruit weight (g)	Length (cm)	Width(cm)	Pulp content (%)	No. Seeds/fruit
Fruit weight (g)	1	0.655*	0.833**	-0.562	0.715
Length (cm)		1	0.596	-0.249	0.512
Width (cm)			1	-0.503	0.595
Pulp content (%)				1	0.658*
No. Seeds/fruit					1

Table 2: Correlation analysis of phenotypic characteristic of *M. holstii* fruits from Rwanda

## Chemical characteristics

pH, titratable acidity(TA) and total soluble solids (TSS) are important chemical parameters for fruit pulp as they determine its quality. The pH of *M. holstii* pulp ( $3.38\pm 0.04$ ) in this study (Table 3) can be regarded as low and acidic in nature. This is also reflected by its high titratable acidity. The ratio of TSS: TA indicates that *M. holstii* has acidic properties which are important in preservation and contributing to its sour taste. Such fruits with acidic properties like that of *M. holstii* are recommended for production of juice and jam.

Parameter	<i>M. holstii</i>	RDA <sup>1</sup>	%RDA	Mango <sup>2</sup>	%RDA-	RD	%RD	%RDA-
					Mango (4-	A <sup>2</sup>	A (19-	Mango (19-
					8yrs)		50yrs	50yrs
							Female	Female)
					(4-8yrs)		a)	
<b>Chemical characteristics</b>								
Titrate acid (TA) (mg/100g)	2.67±0.21							
pH	3.38±0.04							
Total soluble solid (TSS)	13.6±0.1							
Ratio of TSS: TA	5.0							
<b>Proximate composition</b>								
Moisture content (g/100g)	85.33±1.52			78.9-82.8				
Total ash (g/100g)	3.25±0.37							
Total fat	1.50±0.71			0.30-0.53				
Crude protein(g/100g)	8.03±0.95	19	42.3	0.36-0.40	1.9-2.1	46	17.5	0.8-0.9
Dietary fibre(g/100g) DM	25.64±0.06	25	102.6	0.85-1.06	3.4-4.2	25	102.6	3.4-4.2
Beta carotene (mg/g)	0.933±0.014	0.4	233.3	0.003-0.129	0.8-32.3	0.7	133.3	
Vitamin C (mg/100g)	19.80±2.13	25	79.2	13.2-92.8	52.8-371.2	90	22.0	14.7-103.1
Total Carbohydrates (g/100g)	4.33±0.62	130	3.3	16.20-17.18	12.5-13.2	130	3.3	12.5-13.2
Energy (Kcal)	62.94±0.08	2300	2.7	62.1-190.0	2.7-8.3	2900		2.1-6.6
<b>Mineral (mg/100g)</b>								
Sodium	10.066±0.512	1200	0.9	0.0-3.0	0.0-0.3	1500	0.7	0.00-0.02
Potassium	168.12±4.19	3800	4.4	120-210	3.2-5.5	4700	3.6	2.6-4.5
Calcium	10.185±0.132	1000	1.0	7-16	0.7-1.6	1000	1.0	0.7-1.6
Iron	16.262±0.576	10	162.6	0.09-0.41	0.9-4.1	18	90.3	0.5-2.3
Copper	0.573±0.011	0.4	143.3	0.04-0.32	10.0-80.0	0.9	63.3	4.4-35.6
Zinc	2.327±0.034	5	46.4	0.06-0.15	1.2-3.0	8	29.1	0.8-1.9
Magnesium	43.272±0.102	130	33.3	8.0-19.0	6.2-14.6	320	13.5	2.5-5.9
Na/K ratio	0.059							
<b>Bioactive Components</b>								
Total flavonoids (mg/100g as rutin)	9.06±0.64							
Total phenolic compounds (mg/100g) GAE	995.19±48.43			66.02				
Total Anthocyanins (mg/100g pigment)	0.37±0.13							
Antioxidant as IC <sub>50</sub> mg/g AAE	8.49±0.01							

**Table 3:** Chemical characteristics of *M. holstii* fruit pulps of Rwanda. AAE- Ascorbic acid equivalent, GAE – Garlic Acid Equivalent; IRDA<sup>1</sup> (Recommended Daily Allowances based on children 4-8 years old ; RDA<sup>2</sup> : Recommended Daily Allowances based 19-50yrs Females old

\* Maldonado-Celis et al., (2019),

## Proximate Composition

The pulp of *M. holstii* contained crude protein ( $8.03 \pm 0.95$  g/100g) and dietary fibre ( $25.64 \pm 0.06$  g/100g). Therefore, 100g of *M. holstii* in this study can contribute 42% of the RDA for proteins for children of 4 - 8 years and 18% for adults of 19 - 50 years, and 103% of dietary fibre for both children and adults (National Academy of Science, 1989). This makes *M. holstii* fruit pulp a good source of both plant protein and fibre, respectively. Nutritionally, proteins are known to reduce risk of stunting and diseases like Kwasiakow and marasmus. Dietary fibre is essential in aiding absorption and re-absorption of cholesterol and bile acid, lowering incidence of constipation, preventing obesity and development diabetes, and reduce chances of developing colon and breast cancer (Cogoi *et al.*, 2013)

The vitamin C of *M. holstii* pulp ( $19.80 \pm 2.13$  mg/100g) was remarkable. Nutritionally, 100g of the pulp can contribute 79% and 22% of the RDA for vitamin C for children 4-8 years and 19-50 female adults respectively. Vitamin C acts as an antioxidant in the diet, and may reduce the risk of arteriosclerosis, cardiovascular diseases and cancer (Lim *et al.*, 2007; Chalise *et al.*, 2010; Isabelle *et al.*, 2010). The pulp of *M. holstii* also contained  $\beta$ - carotene ( $0.933 \pm 0.014$ mg/100g) that was exceptionally high since 100g of pulp can contribute 233% and 133% of the RDA for beta carotene RDA for 4-8 years and 19-50 adults, respectively (Table 3).  $\beta$ - carotene has ability to convert to retinal (vitamin A) that is associated with reducing risk of developing cancer, age-related macular degeneration, cataracts, and cardiovascular diseases (Wang *et al.*, 2007; Tarwadi and Agte, 2007).  $\beta$ - carotene also functions in gene activation, inflammation and immune processes (Setiawan *et al.*, 2001). Since in most developing countries, vitamin A deficiency is a serious public health concern, *M. holstii* can provide a cheap alternative source of vitamin A.

The concentration of iron ( $16.262 \pm 0.576$  mg/100g) in the pulp of *M. holstii* was exceptionally high because 100g of pulp is able to meet 163 % and 90% of the RDA for iron for 4-8 year and 90% in female adults respectively. Iron is a constituent of hemoglobin, myoglobin, and a number of enzymes that play a role of transporting oxygen from the lungs to the cells in the body (Shetty, 2010). Deficiency of iron is associated with anemia in children and expectant mothers (National Academy of Sciences, 1989). Therefore, diet with *M. holstii* fruit can be used to replace lost iron in female due to menstruation and malaria infected children (Shetty, 2010).

Zinc concentration ( $2.327 \pm 0.034$  mg/100g) reported in the pulp of *M. bolstii* in this study was generally high as 100g of pulp can nutritionally meet 46% and 29% of the RDA for 4-8 year and female adults (19-50 years). Zinc plays an important role in the body in DNA synthesis and replication, cell division and cell growth and differentiation; body defense mechanism and an anti-oxidant. Zinc deficiency is reported to be associated with loss of appetite, growth retardation, skin changes, immunological abnormalities, hypogonadism and dwarfism (National Academy of Sciences, 1989) and increased risk of infections (Shetty, 2010), Therefore *M. bolstii* fruit pulp can improve the health of people by preventing infection and number of health conditions associated with zinc deficiency.

The copper concentration ( $0.573 \pm 0.011$  mg/100g) in the pulp of *M. bolstii* pulp (Table 3) can contribute 143% and 63% of the RDA for 4-8 years and adults (19-50 years), respectively. Although copper is considered a toxic element, its concentration at low concentration is essential for human nutrition. copper functions as a cofactor in cellular metabolic reactions and copper-dependent enzyme catalytic reactions that involve molecular oxygen species (Shajib *et al.*, 2013)

### Bioactive Compounds

The pulp of *M. bolstii* was found to contain phytochemicals ingredients with biological activity like volatile oil compounds, reducing compounds, saponins, alkaloids, anthracenocides, coumarins, sterols and triterpenes, flavonosides and anthocyanocides. Quantitative analysis of bioactive compounds showed that the total phenolic compounds, total flavonoids, total anthocyanins and antioxidant activity were  $995.19 \pm 48.43$  (mg/100g GAE),  $9.06 \pm 0.64$  mg/100g as rutin,  $0.37 \pm 0.13$  mg/100g pigment and  $8.49 \pm 0.01$  mg/g AAE respectively (Table 3). The total phenolic value of *M. bolstii* (995 mg/100g) can be have biological activities including antimicrobial, anti-inflammatory (Chalise *et al.*, 2010), anti-cancer (Ajila *et al.*, 2007) and anti-hypertensive and anti-diabetic (Isabella *et al.*, 2010). With the growing interest for natural antioxidants (Huang *et al.*, 2004), *M. bolstii* fruit can be an alternative fruit source for preventing age related diseases like cancer and cardiovascular diseases.

### Physico-chemical characteristics of Seed oil

Apart from the fruit pulp, fruits seeds are also very important in extraction of vegetable oil for food, cosmetic,

pharmaceutical and biofuel applications. More often than not, seeds of *M. bolstii* are normally discarded after eating the pulp. In this study, the seed of *M. bolstii* had the oil yield of  $37.67 \pm 1.53\%$ , liquid oil at room temperature with yellow-orange colour (Table 4). This finding was 35.2% *M. bolstii* seed oil yield that was reported by Mitzangi *et al.*, (2011). The oil yield of *M. bolstii* seed was considerably high enough to make it suitable crop for vegetable oil production. Plant seeds with about 20% or more oil yield content are suitable for commercial production of vegetable oil (Varzakas and Tzia, 2015).

The physical and chemical characteristics of *M. bolstii* seed oil in this study were within the United National Food and Agricultural Codex Alimentarius standards for vegetable oil (El-Adawy and Taha, 2001; Prescha *et al.*, 2014). Analysis of fatty acid profile showed five major acids with omega 6 (linoleic fatty acid) being the most dominant.

Parameter	Result
oil yield	$37.67 \pm 1.53$
Colour	Yellow-orange
State at room temperature	Liquid
Refractive index	$1.476 \pm 0.000$
Acid value (mgKOH/kg)	$2.48 \pm 0.17$
Free fatty acids	$1.24 \pm 0.08$
<b>Fatty acids (%)</b>	
Palmitic (16:0)	$4.77 \pm 0.19$
Stearic (18:0)	$4.51 \pm 0.73$
cis-vaccenic (18:1n7)	$1.64 \pm 0.13$
Oleic (18:1n9)	$10.15 \pm 1.18$
Linoleic (18:2n6)	$78.92 \pm 3.24$
Ratio Oleic: linoleic	0.13
Total Saturated fatty acid (SFA)	9.28
Total Monounsaturated fatty acids (MUFA)	11.79
Total Poly unsaturated fatty acids (PUFA)	78.92
Total Un saturated fatty acids (UFA)	90.71
Ratio PUFA: SFA	8.50

Table 4: Physico-chemical characteristics of *M. bolstii* seed oil of Rwanda

In fact, 78% linoleic acid in *M. bolstii* seed oil was comparable to value of 80.2% report for *M. bolstii* seed oil samples from the Democratic Republic of Congo as reported by Minzangi *et al.* (2011). Nutritionally, linoleic acid is important in the prevention of cardiovascular disorders, atherosclerosis, cancer and hypertension.



The total unsaturated fatty acids (UFA) in *M. holstii* seed oil was 90.91% with the total poly unsaturated fatty acids (PUFA) being 78.92%. The high level of un-saturation in *M. holstii* oils demonstrated by ratio of PUFA: SFA (8.5:1.0) has health promoting properties of preventing cardiovascular diseases and lowering serum cholesterol in the body.

## Conclusion

*Myrianthus holstii* fruit pulp is rich in micro nutrients like iron, zinc, copper and beta carotene, and the seed oil in omega 3. These nutrients are essential in nutrition and health. Therefore, fruits can be consumed as a snack or processed into food products like fruit juice, wine, jelly, jam and vegetable oil can enhance nutrition, health and income of most households. The nutrients from *M. holstii* fruit can be used to supplement micronutrient deficient staple foods like plantain, cassava, potatoes and sorghum. Furthermore, the establishment of a processing facility for *M. holstii* fruit into juice, jam and wine, farmers with fruits can generate additional income by selling their harvest.

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## References

Ajila, C., Naidu, K., Bhat, S., & Rao, U. (2007). Bioactive compounds and antioxidant potential of mango peel extract. *Food Chemistry*, 105(3), 982-988.

AOAC. (1997). Association of Official Analytical Chemists. Official methods of analysis. 17th ed. Washington, DC.

Bigirimana, C., Omujal, F., Isubikal, P., Bizuru, E., Obaa, B., Malinga, M., Agea, J.G. & Lamoris, J. B. (2016). Utilisation of indigenous fruit treespecies within the Lake Victoria Basin, Rwanda. *Agr Sci Int J*, 1, 1-13.

Borkatoky, M., Kakoakoty, B., & Sakia, L. (2013). Influence of total phenolic content and total flavonoid content on the DPPH radical scavenging activity of *Eclipta alba* (L.) Hassk. *Int. J. Pharm. Pharm. Sci*, 5, 224-327.

Chalise, J.P., Acharya, K., Gurung, N., Bhusal, R.P., Gurung, R., Skalko-Basnet, N. & Basnet, P. (2010). Antioxidant activity and polyphenol content in edible Wild fruits from Nepal. *International journal of food sciences and nutrition*, 61(4): 425-432.

Cogoi, L., Giacomino, M. S., Pellegrino, N., Anesini, C., & Filip, R. (2013). Nutritional and phytochemical study of *Ilex paraguayensis* fruits. *Journal of Chemistry*, 750623 (2013), 1-6. <http://dx.doi.org/10.1155/2013/750623>.

Culei, I. (1982). Methodology for the analysis of vegetable drugs. Practical Manual on the industrial Utilisation of Medicinal and Aromatic Plants. *Center Building, University of Bucharest, Romania*, 67-81.

Demirsoy, H. & Demirsoy, L. (2004). A study on the relationships between some fruit characteristics in cherries. *Fruits* 59; 219-223.

El-Adawy, T. A., & Taha, K. M. (2001). Characteristics and composition of watermelon, pumpkin, and paprika seed oils and flours. *Journal of agricultural and food chemistry*, 49(3), 1253-1259.

Emaldi, U., Trujillo, I., & De-García, E. (2004). Comparison of characteristics of bananas (*Musa* sp.) from the somaclone CIEN BTA-03 and its parental clone Williams. *Journal of Fruits*, 59, 257-263.

Francis F.J. (1982). Analysis of anthocyanins. In: Markakis P, editor. Anthocyanins as food colors. London (UK):Academic Press. p. 181-207.

Franquin, S., Marcelin, O., Aurore, G., Reynes, M., & Brillouet, J-M. (2005). Physicochemical characterisation of the mature-green Golden apple

(*Spondiascythera sonnerat*). *Journal of Fruits*, 60, 203–210

Huang, H-Y., Chang, C-K, Tso, T. K., Huang, J-J., Chang, W.W., & Tsa, Y-C. (2004). Antioxidant activities of various fruits and vegetables produced in Taiwan *International Journal of Food Sciences and Nutrition*, 55, (5), 423-429.

Isabelle, M., Lee, B. L., Lim, M. T., Koh, W.-P., Huang, D., & Ong, C. N. (2010). Antioxidant activity and profiles of common fruits in Singapore. *Food Chemistry*, 123(1), 77-84.

Kamungu, S., Basabose, K., Bagalwa, M., Bagalwa, B., Murhabale, B., & Yamagiwa, J. (2015). Phytochemical Screening of Food Plants Eaten by Sympatric Apes (*Gorilla beringei graueri* and *Pan troglodytes schweinfurthii*) Inhabiting Kahuzi-Biega National Park, Democratic Republic of Congo) and their Potential Effect on Gastro Intestinal Parasites. *International journal of pharmacognosy and phytochemical research*, 7(2), 255-261.

Katende, A. B., Birnie, A., & Tengnas, B. (1995). Useful trees and shrubs for Uganda: Regional Soil Conservation Unit.

Lim, Y., Lim, T., & Tee, J. (2007). Antioxidant properties of several tropical fruits: A comparative study. *Food Chemistry*, 103(3), 1003-1008.

Magadula, J. J., & Tewtrakul, S. (2010). Anti-HIV-1 protease activities of crude extracts of some *Garcinia* species growing in Tanzania. *African Journal of Biotechnology*, 9 (12).

Maldonado-Celis, M. E., Yahia, E. M., Bedoya, R., Landázuri, P., Loango, N., Aguillón, J., Restrepo, B. & Ospina, J. C. G. (2019). Chemical composition of mango (*Mangifera indica* L.) fruit: nutritional and phytochemical compounds. *Frontiers in Plant Science*, 1073 (10), 1-21 doi: 10.3389/fpls.2019.01073

Matsumoto-Oda, A., & Hayashi, Y. (1999). Nutritional aspects of fruit choice by chimpanzees. *Folia Primatologica*, 70(3), 154-162.

Maundu, M., Ngugi, W., & Kabuye, H. (1999). *Traditional food plants of Kenya*: Resource Centre for Indigenous Knowledge (KENRIK). National Museums of Kenya.

Minzangi, K., Kaaya, A. N., Kansime, F., & Samvura, J. R. T. B. (2011). Oil content and

physicochemical characteristics of some wild oilseed plants from Kivu region Eastern Democratic Republic of Congo. *African Journal of Biotechnology*, 10(2), 189.

Moo-Huchin, V. M., Moo-Huchin, M. I., Estrada-León, R. J., Cuevas-Glory, L., Estrada-Mota, I. A., Ortiz-Vázquez, E., Betancur-Ancona D. Sauri-Duch, E. (2015). Antioxidant compounds, antioxidant activity and phenolic content in peel from three tropical fruits from Yucatan, Mexico. *Food Chemistry*, 166, 17-22.

Naluswa, J. (1993). A report on a pilot country study of non-wood forest products in Uganda. *Commonwealth Science Council. Commonwealth Secretariat. London.*

National Academy of Science (1989): Recommended dietary allowances (10<sup>th</sup> edition). Food and Nutrition Board. National Academy Press. Washington DC pp 1-298  
National Institute of Statistics Rwanda (2013). Statistical year book 2013. <https://www.statistics.gov.rw/publication/statistical-yearbook-2013>

Ogwang, P., Nyafuono, J., Agwaya, M., Omujal, F., Tumusiime, H., & Kyakulaga, A. (2011). Preclinical efficacy and safety of herbal formulation for management of wounds. *African Health Sciences*, 11(3).

Okullo, J.B.L., Omujal, F., Agea, J.G., Vuzi, P.C., Namutebi, A., Okello, J.B.A. & Nyanzi, S.A. (2010a). Proximate and mineral composition of shea (*Vitellaria paradoxa* C.F. Gaertn) fruit pulp in Uganda. *African Journal of Food, Agriculture Nutrition and Development* 10 (11), 4430-4443.

Okullo, J. B. L., Omujal, F., Agea, J., Vuzi, P., Namutebi, A., Okello, J., & Nyanzi, S. (2010b). Physico-Chemical characteristics of shea butter (*Vitellaria paradoxa* CF Gaertn.) oil from the Shea district of Uganda. *African Journal of Food, Agriculture, Nutrition and Development*, 10(1).2070-2084

Oomah, B. D., Busson, M., Godfrey, D. V., & Drover, J. C. (2002). Characteristics of hemp (*Cannabis sativa* L.) seed oil. *Food Chemistry*, 76(1), 33-43.

Prescha, A., Grajzer, M., Dedyk, M., & Grajeta, H. (2014). The antioxidant activity and oxidative stability of cold-pressed oils. *Journal of the American Oil Chemists' Society*, 91(8), 1291-1301.

Reiner, W. B., Petzinger, C., Power, M. L., Hyeroba, D., & Rothman, J. M. (2014). Fatty acids in mountain gorilla diets: implications for primate nutrition and health. *American Journal of Primatology*, 76(3), 281-288.

Rode, K. D., Chiyo, P. I., Chapman, C. A., & McDowell, L. R. (2006). Nutritional ecology of elephants in Kibale National Park, Uganda, and its relationship with crop-raiding behaviour. *Journal of tropical ecology*, 22(04), 441-449.

Rodriguez-Amaya, D. B., & Kimura, M. (2004). HarvestPlus handbook for carotenoid analysis (Vol. 2): International Food Policy Research Institute (IFPRI) Washington. *Harvest Plus Technical Monograph 2*. 63pp.

Rothman, J. M., Dierenfeld, E. S., Molina, D. O., Shaw, A. V., Hintz, H. F., & Pell, A. N. (2006). Nutritional chemistry of foods eaten by gorillas in Bwindi Impenetrable National Park, Uganda. *American Journal of Primatology: Official Journal of the American Society of Primatologists*, 68(7), 675-691.

Rothman, J. M., Plumptre, A. J., Dierenfeld, E. S., & Pell, A. N. (2007). Nutritional composition of the diet of the gorilla (*Gorilla beringei*): a comparison between two montane habitats. *Journal of tropical ecology*, 23(6), 673-682.

Seal, T. (2012). Antioxidant activity of some wild edible plants of Meghalaya state of India: A comparison using two solvent extraction systems. *International Journal of Nutrition and Metabolism*, 4(3), 51-56.

Setiawan, B., Sulaeman, A., Giraud, D. W., & Driskell, J. A. (2001). Carotenoid content of selected Indonesian fruits. *Journal of Food Composition and Analysis*, 14(2), 169-176.

Shahbazi, F., & Rahmati, S. (2013). Mass modeling of fig (*Ficus carica* L.) fruit with some physical characteristics. *Food science & nutrition*, 1(2), 125-129.

Shajib, M. T. I., Kawser, M., Miah, M. N., Begum, P., Bhattacharjee, L., Hossain, A., Formsgaard, I.S., Islam, S. N. (2013). Nutritional composition of minor indigenous fruits: Cheapest nutritional source for the rural people of Bangladesh. *Food Chemistry*, 140(3), 466-470.

Shetty, P. S. (2010). *Nutrition, immunity and infection*. CABI. Wallingford, Oxfordshire, UK, pp 88-110

Shiban, M. S., Al-Otaibi, M. M., & Al-Zoreky, N. S. (2012). Antioxidant Activity of pomegranate (*Punica granatum* L.) fruit peels. *Food and Nutrition Sciences*, 3(7), 991.

Simirgiotis, M. & Schmeda-Hirshmann. (2010). Determination of phenolic composition and antioxidant activity in fruits, rhizomes and leaves of the white strawberry (*Fragaria Chiloensis* spp. Chiloensis form chiloensis) using HPLC-DAD-ESI-MS and free radical quenching techniques. *Journal of Food composition* 23, 540-544.

Singleton, V., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16(3), 144-158.

Tarwadi, K & Agte, V. (2007). Antioxidant and micronutrient potential of common fruits available in the Indian subcontinent. *International Journal of Food Sciences and Nutrition*, 58(5), 341-349.

Usenik, V., Kastelec, D., & Štampar, F. (2005). Physicochemical changes of sweet cherry fruits related to application of gibberellic acid. *Food Chemistry*, 90(4), 663-671.

Varzakas, T., & Tzia, C. (2015). Handbook of food processing: food safety, quality, and manufacturing processes (Vol. 35). CRC, Press. New York.

Wang, Y.C., Chuang, Y.C., & Ku, Y.H. (2007). Quantitation of bioactive compounds in citrus fruits cultivated in Taiwan. *Food Chemistry*, 102(4), 1163-1171.

Xu, W., Liu, L., Hu, B., Suu, Y., Ye, H., Ma, D. and Zeng, X. (2010). TPC in the leaves of 116 potatoes (*Ipomea batata* L.) varieties and pushu 53 leaf extracts. *Journal of Food Composition* 23, 599-604.