Analysis of Selected Nutrient Levels at Different Growth Stages of *Dovyalis Caffra* (Kei-apple) Fruits

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Abstract: **- The quest to attain food security has led to domestication of the previously-termed wild fruits; amongst them *Dovyaliscaffra* (kei-apple) fruits. Radical human lifestyle changesand change in climatic conditions demands that food should not only be for basic nutrition, but also health benefits. This paper purposed to evaluate the nutrition and health levels of *Dovyaliscaffra* fruits at different growth stages using wet chemistry (Titration, pH, and Kjedhal method) and spectrophotometry (UV-VIS and AAS). For Carbohydrates, sucrose and fructose levels decreased with age as glucose levels increased. Both Proteins and Lipid levels decreased with time. The Iron content increased linearly, from 1.04900 $\pm$ 0.000005ppm in young fruit to 1.15780 $\pm$ 0.000001 ppm in old fruit. While the zinc content increased nonlinearly from0.16384 $\pm$ 0.000002 ppm in young fruit to 0.21523 $\pm$ 0.000009 in old fruit.Copper levels remained fairly constant as the fruits aged (0.01430 $\pm$ 0.000007 ppm) and in cobalt the concentration decreased from 0.05604 $\pm$ 0.000005 ppmto 0.03199 $\pm$ 0.000006. Only Green (young) Kei-apple fruits indicated positive antioxidant scavenging capacity against DPPH radical at 515nm with an IC_{50} level of 28.1385 ± 3.2224 µg/mL.**

***Key words*: Food security, *Dovyaliscaffra*, nutrition and health levels.**

I. INTRODUCTION

The search for new and diverse foods have intensified to meet an ever increasing human and animal population. Food security bodies such as Food and Agriculture Organization, FAO aim at providing adequate, safe food and water not only presently but also in the future. The World Food Summit of 1996 defined food security as existing "when all people at all times, have physical and economic access to sufficient, safe and nutritious food preferences for an active and healthy life"[1]

Figure 1: Kei apple fruits(L) and Kei apple tree (R).

The horticulture industry, especially vegetables and fruit producers is of growing interests to agriculture entrepreneurs of various scales in low-and-middle income countries. Fruits are important sources of vitamins, folates, fibers, minerals and phytochemicals. It is worth noting that accessibility of fruits is a key factor to its Vegetables and Fruits (V &F) nutritional contribution. Wild fruits and berries are mostly considered inedible or even toxic unless proved otherwise; historically or scientifically by analysis. Kei apples (*Dovyaliscaffra*) are an example of wild fruits whose edibility in Africa depend with the corresponding community beliefs. Some communities regard the fruit edible while others inedible.

Fruits are considered the primary source of vitamins, phenolic acids and minerals and the secondary sources of fibres and carbohydrates. The three basic macronutrients include lipids, carbohydrates and proteins. Different fruits have varying carbohydrate concentrations with berries having the lowest percentage of digestible carbohydrates (5-12%) [2]. For majority of healthy individuals, normal blood sugar levels range between 72-108mg/dL when fasting and 140mg/dL two hours after a meal [3]. Above or below these values, people are susceptible to diabetes [4]. Fruits are usually ingested to regulate carbohydrate levels in the blood. Diets containing low digestible carbohydrate levels (net carbs/fibre is not counted) are regarded as keto low-carb diets (20g per day) and individuals consuming them are advised to consume more grains and berries for fruits. Diets yielding 20-50g per day are regarded as moderate low-carb diets and those consuming them are advised to take a fruit daily. Diets with 50-100g per day of carbohydrates are liberal carb-diets and those taking them require at least two or three fruits daily to aid regulate the starch levels [5].

Together with vegetables, fruits produce very low lipids and fatty acids. Some of these lipid macromolecules are found in the fruit tissues while most occur on the skin in form of wax [6]. Protein levels from most fruits are also quite low. The human body requires 8g of protein for every 20 pounds of body weight. Proteins are mainly produced by legumes and animal products and are a very crucial macronutrient whose deficiency cause Kwashiorkor. Most protein sources from fruits, vegetables and grains lack one or more essential amino acid [7]. Fruits contain phenolic acid in large amounts. These acids are natural antioxidants, both in vivo and in vitro and

thus make fruits a major antioxidant source. Fruits are thereby ingested to prevent radical free compounds that cause wrinkling of the skin and ultimately aging. Fruits are a major source of essential bio minerals; essential for catalyzing body functions, formation of tissues and hormones, for immunity as well as regulating blood pressure and cholesterol levels.

Kei apple is indigenous to the southern regions of Africa, including Malawi, Zimbabwe, Mozambique and South Africa [8]. Over time, the tree have been domesticated with an increasing populace of its use as a live fence. This is mainly due to its thorny branches and quick growth rate. Being ever-green, it provides a year-round screen while its sharp thorns deter both people and animals [9]. Not only does Kei apple trees tolerate dry soils but also saline soils. Kei apple trees thrive more in drylands, attaining 8-9 metres[10]. Buds at the base of the spine produce clusters of alternately arranged simple ovulate leaves 3-6cm long. Kei apple fruits are bright yellow or orange globose berries when ripe. Beneath the light uniform skin is a juicy pulp; fleshy with dotted white seeds. The pulp is too acidic (sour) due to excess Vitamin C and tartaric acid [8]. Overgrown kei apple fruits appear more fibrous with a characteristic reduced sourness. Fresh Kei apple fruits are rich in Vitamin C (80-120mg/100g) and Potassium (>600mg/100g) [9]. Sugars generally exceed 15% with pectin levels nearly at 4% [9]. The amount of amino acids is quite low [9]thus low protein content. Beyond that, little of these fruits food value is known [9].

The presence and levels of most nutrients significant to humans, in Kei apple fruits is still unknown. Determination of these nutrients and their levels is likely to change people perception concerning it, and possibly cultivate it in large scale as a cash crop. Analysis of the nutrient levels at different growth stages of the fruit is critical in determining the right fruits required for different type of people at different conditions, such as children or lactating women. This work purposed at analyzing the levels of carbohydrates, proteins, lipids, selected biominerals and antioxidants at three distinctive maturity stages of Kei apple fruits.

II. METHODOLOGY

2.1 Requirements

Chemicals; Lab. Grade Sodium hydroxide, Sigma-Aldrich, Universal indicator solution, Merck, 80% Ethyl alcohol, Merck, Whatman # 1 Filter papers, Standard d-ribose, d-fructose, d-lactose, sucrose and soluble starch, all Lab. Grade, Sigma-Aldrich, Anhydrous Copper sulphate, Sigma-Aldrich, Copper sulphate pentahydrate, Sigma-Aldrich, Methyl blue indicator, Alundum granules, Potassium sulphate, 98% pure, sp. gravity 1.84 Sulphuric acid, Sigma-Aldrich, Conc HCl acid, Merck, Methyl red indicator, Sigma-Aldrich, Chloroform, Methanol, Merck, 2,2-Diphenyl-1-picryl hydrazyl (DPPH) solution, Sigma-Aldrich.

2.2 Experimental Design

A randomized block experimental design was used. Samples of Kei-apple fruits from Maasai Mara University (co-ordinates 35.87 ^{0}E & 1.08 ^{0}S) fence hedges and University farm were randomly collected and pooled into baskets. An average of 100 Kei-apple fruits for each fruit stage of size 25-35mm diameter. Sorting was later done to remove fruits infested with worms. The fruits were then classified into three blocks (strata) based on their coloration only i.e. green, yellow and brown. This criterion was prospected to differentiate the fruits ages with green hypothesizing young fruits, yellow hypothesizing medium-aged fruits and brown hypothesizing old fruits. Any fruit in these strata had an equal probability of being picked for characterization or analysis thus subsequent picking from the blocks was by randomization.

2.3 Sampling

Stratified sampling method was used as randomly collected Kei-apple fruits were grouped into strata according to their colors. Samples were then randomly taken from these strata for characterization and nutritional analysis. A dark canvas bag was used during sample collection.

Figure 2: The different stages of Kei apple fruits as they age.

2.4 Sample pretreatment

Removal of debris and any vegetatious matter was done manually. The samples were maintained uncompressed/ slightly separate from each other to minimize chances of inter-tissue transfer and ripening. The samples were maintained in a cool, dry and aerated tray away from direct sunlight to minimize any reactions with confounding variables. Fruit sepals remained intact to avoid piercing the fruits while plucking them out.

2.4.1 Sample preparation

Before characterization or analysis, the fruits of a particular strata were mushed together using a mortar and pestle and their seeds and skin gently peeled out using a pair of forceps. The remaining flesh was mushed until syrup was obtained. Syrups of different fruit strata were stored in 250 ml plastic bottles separately not to come into contact with each other.

2.5 Sample characterization

2.5.1 pH

The pH of each of the three samples was determined using a pH meter, Hanna G114, after calibration with buffers pH 7.00, pH 4.00 and pH 9.00.

2.5.2 Total Solids (TS)

Sample syrups of predetermined volumes were put on a pre-weighed ceramic crucible. 5.00g of samples were then heated slowly at 55^0C for 3 and a half hours and the mass of the crucible contents again taken after cooling to 20^0C in an oven (Shimadzu). This was done in triplicates and the average TS calculated.

$\%TS = (W_{INITIAl} - W_{FINAl})/ W_{INITIAL}$

2.5.3 Volatile Fatty Acids (VFAs)

Sample syrup (80.0 ml) was distilled using distilled water before transferring to a burette and finally used to titrate 10.0ml of 0.1N NaOH solution from pH 13.0 to pH 8.3. An accurate Universal indicator solution was used. The Average Titres were recorded and used to calculate the % VFAs in the samples.

2.6 Carbohydrate determination by Lane-Eynon method

Sample syrup were boiled in 80% ethanol solution (2:1) to defatten. The boiled solution was then filtered using Whatman # 1 filter paper and both the filtrate and retentate retained. Both fractions were then dried and weighed to determine their concentration. Alcohol was then removed by refluxing at 78^0C in a water bath. To remove other soluble substances in the filtrate (e.g organic acids, amino acids, pigments, vitamins and minerals), clarification using lead acetate trihydrate as a clarifier. The Lane- Eynon titration method was then used analyze the levels of carbohydrates in each of the samples and the concentrations obtained fitted in a Standard carbohydrate calibration curve as explained below;

10.0 ml of 0.5M $CuSO_4.5H_2O$ solution plus 2-3 drops of Methylene blue indicator was put into a round bottomed flask and gently boiled. A standard carbohydrate solution was slowly added from a burette and the Av. Titre taken to change the color of the boiling solution from blue to white recorded. Different carbohydrate standards i.e. D-Ribose, D-Fructose, Lactose, Sucrose and Soluble starch were all used and a standard Calibration curve formulated from their concentrations obtained after titrating with boiling $CuSO_4$ solution.

The titration process was then repeated using Kei-apple fruit samples instead of standard carbohydrate solutions and the concentrations obtained fitted into the Calibration curve formulated. The carbohydrate in the mixture sample correlating to a certain standard is speculated to have more concentration of that standard. For example, if the young fruits give titrimetric values closer to those of d-ribose, they were assumed to have high ribose concentration. FTIR analysis was further done to affirm these findings.

2.7 Protein analysis by Kjedhal process

Kjedhals Nitrogen analysis method was used to determine the amount of nitrogen in the samples before multiplying with Crude Protein factor to get protein content.

1.00g of ground sample was put in a round bottomed flask and 15g of K_2SO_4, 0.004g Anhydrous $CuSO_4$ and 0.5g alundum granules added. 20.0ml of concentrated H_2SO_4 was then added and the mixture heated until white fumes cleared off the bulb of the flask, swirled gently and continued heating for 30 more minutes. After cooling, 250ml of distilled water was slowly added through the sides of the flask. 80ml of 45% NaOH solution was then added without shaking and the flask connected to a distillation apparatus. The mixture was then distilled and the distillate collected into 85ml of 21.4% HCl solution as the trapping reagent.

The excess base was then back-titrated against standard HCl solution (0.1N) using Methyl red indicator and the Average titre taken to change the color to orange recorded.

These procedures were then repeated for the two other samples and a blank that had no sample at all.

Moles Ammonia = moles Acid – moles Base

$$(M_{Acid} * Vol._{flask}) – (M_{Base} * Vol._{burette})$$

$Gms_{Nitrogen}$ = moles Ammonia * A.m.u (14.0067)

$\%$ Nitrogen = $Gms_{Nitrogen} / gms_{Sample} * 100$

% Crude Protein (CP) calculation; CP (Dry matter) = % N (DM) * F

*For fruits, F = 4.3

2.8 Determination of Lipids by Bligh and Dyer method

0.500g of wet Kei-apple fruit flesh samples were weighed and homogenized for 2 minutes with 5ml chloroform and 5ml methanol. To the homogenized mixture, 5ml of chloroform was added and the mixture again homogenized again for 30 seconds. 5ml of water was added to the mixture and the sample homogenized for another 30 seconds. The mixture was then allowed to separate and the lower solvent phase removed and passed through a Whatman # 1 filter paper and the filtrate preserved in a labelled vial.

Another 5ml of chloroform was added to the remaining pellet and aqueous phase and homogenized again for 2 minutes. The resultant mixture was added to the previous filtrate by passing it through the Whatman # 1 filter paper. The filtrate was allowed to separate in another graduated cylinder or burette and the volume of the lower chloroform recorded. Lipids were then gravimetrically determined by placing 0.5ml aliquots of the chloroform layer into pre-weighed aluminium pans (3 pans per sample), allowing the samples to evaporate overnight, recording the weights and converting them to percent lipids.

2.9 Determination of levels of Essential bio minerals (micronutrients)

Samples were digested using aqua regia solution (1.0g of sample in 20ml of acid), filtered using Whatman # 1 filter paper, diluted to 100ml using distilled water then a drop of

1% HNO_3 added for preservation in a plastic container at -4^{0}C in a refrigerator.

Standards of Fe^{2+}, Cu^{2+}, Zn^{2+} and Co^{3+} were prepared from their respective AAS grade salts beginning with a 1000ppm stock solution before serial dilution to required concentrations of 0.2ppm, 0.4ppm, 0.6ppm, 0.8ppm, 1.0ppm and 1.2ppm for all ions except Fe^{2+} which had 2.0ppm as its last standard concentration.

After standard analysis by Atomic Absorption Spectrophotometer, AAS (Shimadzu 6800), samples in triplicates were analyzed. A threshold correlation factor of r^2 = 0.985 was maintained for absorbance against standard concentrations before any sample analysis was attempted. A blank correction was done after every set of triplicate sample analysis.

2.10 Determination of Antioxidant levels by UV-VIS

2,2-Diphenyl-1-picryl hydrazyl (DPPH) solution in methanol (6 * 10^{-5} M) was prepared. 3ml of this solution with 100 microlitres of methanolic solutions of plant extracts were mixed. The samples were then incubated in a water bath at 37^{0}C for 20 minutes. The decrease in absorbance at 515nm was measured (A_E) (DPPH radicals have a maximum absorbance at 515nm which disappear with reduction by antioxidant compounds). The authentic standards and sample solution were scanned by a UV/Vis spectrophotometer (UV-550; Jasco, Japan) at 515nm to inspect their absorbance.

The experiment was carried out in triplicates;

% inhibition = $(A_B - A_E)/A_B * 100$

Where A_B = Absorbance of blank and A_E = Absorbance of plant extract

2.11 Statistical analysis

All statistical analysis for the means, standard deviations and variance were done using MS Excel while Correlation and regression, root mean square values, f-test and significant difference between various data sets of the fruits were done using One-Way ANOVA incorporated in Originlab 6.1 software.

III. RESULTS AND DISCUSSION

3.0 Characterization

The pH was found to decrease as the VFAs were increasing with age of the fruits. There was no specific trend in the Total Solid content of the fruits.

Table 1: Characterization of different fruits syrups used.

Fruit age	Mass of crude analyte (g)	Average pH	Average TS	Average VFAs
Young	30.00 ± 0.02	2.98 ± 0.031	12.33 ± 0.067%	3.312 ± 0.0444%
Middle aged	30.00 ± 0.02	2.55 ±0.012	11.87 ± 0.054%	3.471 ± 0.0237%
Old	30.00 ±0.02	1.16 ±0.041	13.11 ± 0.011%	3.884 ± 0.0021%

3.1 Analysis of Carbohydrate Levels

The average Titres of standard 0.1M carbohydrate solutions and samples when titrated against 5.0ml of boiling 0.125M $CuSO_4.5H_2O$ are indicated below;

Table 2: Average volumes used during Lane-Eynon Titration of Standard Carbohydrates and Samples against Copper (II) Sulphate titrand.

Standards	Molecular Weight (mol^{-1})	Av. Titre (ml)
D-ribose	150.000	18.80 ± 0.1
D-fructose	180.156	16.00 ± 0.1
Lactose	342.300	0.50 ± 0.1
Sucrose	342.300	2.50 ± 0.1
Soluble starch	342.300	3.00 ± 0.1
SAMPLES		
Young Kei-apples		1.45 ± 0.1
Medium-aged Kei-apples		2.18 ±0.1
Old Kei-apples		3.53 ± 0.1

The average values obtained above were found to be significantly different at 95% confidence level (n=14)

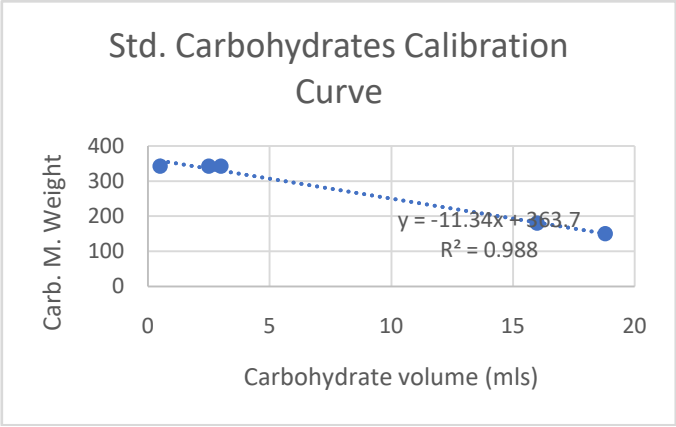


Figure 3: Calibration graph of Standard carbohydrates

Following the above Calibration graph and by interpolation of the sample values, the Young, Medium-aged and Old Kei apple fruits were found to have Sucrose and fructose for the Old fruits. The data was confirmed using the FTIR spectra below;

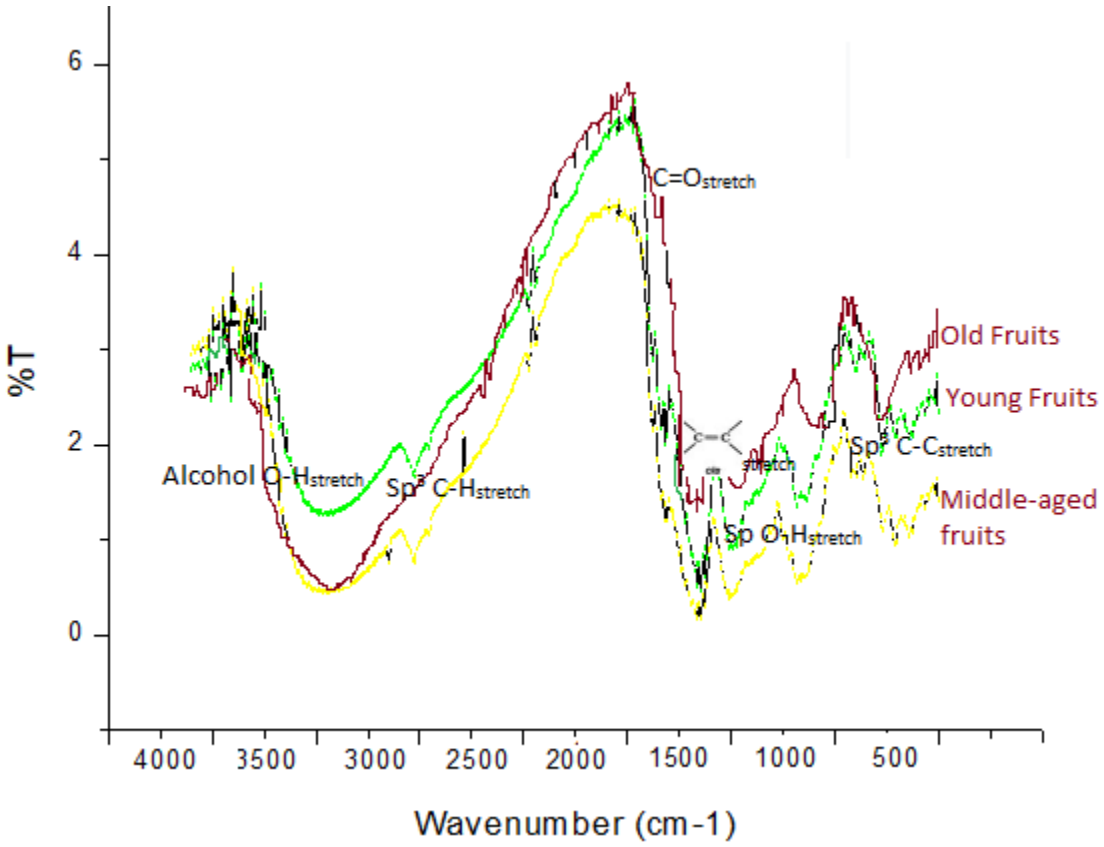


Figure 4: FTIR Spectra of the different Fruit samples analyzed.

The FTIR spectra of the samples had Functional Group signatures corresponding to those of Sucrose and Fructose for the Medium-aged and Old fruits. Lactose yielded an almost negligible amount further attesting its rare occurrence in plant organs. There was a general increase in analyte solution as the Kei apple fruits age progressed indicating increased carbohydrates with fruit age. While the Young/Green fruits (S-1) showed little if any carbohydrate, the medium aged/Yellow samples (S-2) indicated values close to those of Standard Sucrose. The older/Brown samples (S-3) had values close to those of soluble starch. Young plant organs are known to contain less carbohydrates but more proteins since they require to grow. The increase in starch with decreasing sucrose in Kei apple fruits over time can be attributed to sucrose hydrolysis.

$$sucrose \rightarrow glucose + fructose$$

The hydrolysis of sucrose is spontaneous, occurring naturally in plant organs as they age. While the enzyme sucrase is required, acidic conditions accelerate the reaction [11]. This works optimally for Kei apples which are naturally acidic. Tartaric acid present in the fruits enhance the reaction further by breaking down acetal (glycosidic) bonds present. Thus, old Kei apples have little sucrose and more glucose levels. Another potential source of the increased glucose levels in older fruits would be lactase hydrolysis of lactose to form lactose hydrolytic sugars including glucose, galactose and oligosaccharides. [11, 12].

3.2 Protein Analysis

The results from titration of basic distillate against 10.0ml of 0.1N HCl in Kjedahls process are indicated below. A Crude Protein (CP) factor of 4.3 for fruits was used.

Table 3: Levels of protein in varying fruit samples analyzed.

	Titre readings (ml)	Average titre (ml)	% Nitrogen %N = moles Acid/1000 * (V_s-V_b)/M_g * 14.0067/moles *100	% Protein (N*4.3)
Blank	0.9, 0.8, 0.9, 0.8	0.85 ± 0.1	0.000 ±0.0001	0.000±0.0002
Young Kei apples	5.3, 5.2, 5.4, 5.4	5.33 ± 0.1	0.6275 ±0.0007	2.698±0.0011
Medium-aged Kei apples	5.0, 5.1, 5.0, 5.2	5.08 ± 0.1	0.5924±0.0003	2.547±0.0006
Old Kei apples	4.8, 4.7, 4.7, 4.8	4.75 ± 0.1	0.5462±0.0009	2.349±0.0009

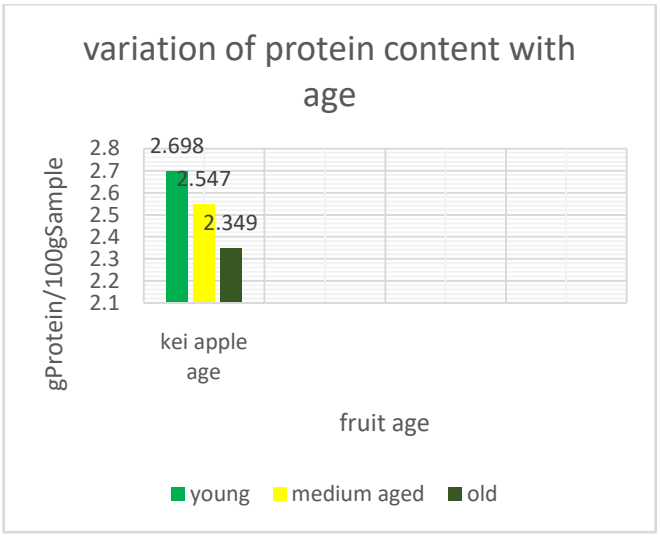


Figure 5: Variation of protein content with age of fruits.

The younger fruits indicated more protein content than the rest with the old fruits indicating the least protein content. The two groups were found to be significantly different at 95% Confidence level (n=9, r^2 = 0.99975, Coefficient of Variation = 0.000109). Protein content in plants is directly related to nitrogen content. As plant organs age, they change in composition due to stress-induced developmental aging or age-related developmental aging. The earlier is more so due to human issues such as pollution while the latter is naturally occurring. Some of these changes involve transition of nutrient minerals and remobilization from older to other newer organs [13]. Nitrogen in plant organs senescence gradually to form new organs since new organs require amino acids for growth. Developing organs thus act as a 'sink' towards which nitrogen efflux to. This senescence of organs led to younger fruits having more protein content than their older counterparts. Another factor that can lead to decreased protein content with age is protein breakdown to amino acid constituents and subsequent resynthesizing of these amino acids which drastically deplete them.

3.3 Lipid Analysis

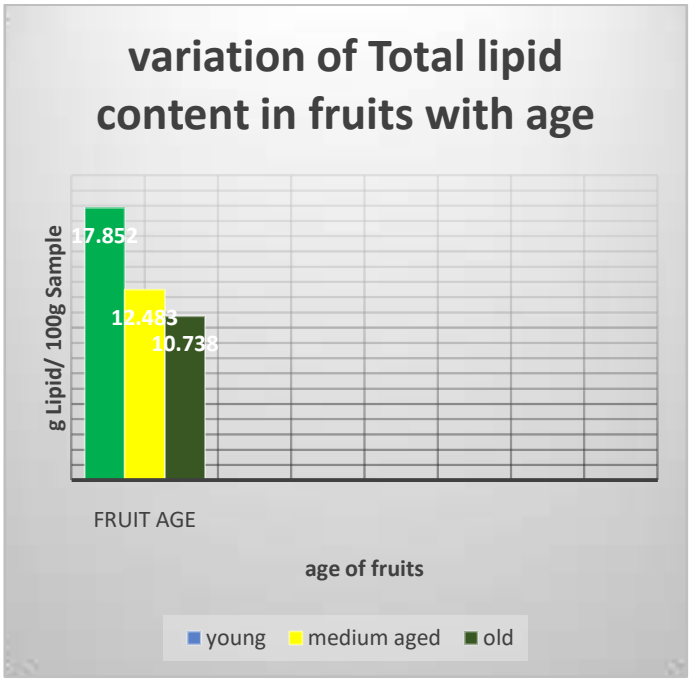
The volumes of chloroform layer and their corresponding gravimetric weights upon air-drying for 12 hours in aluminium pans as described in Dyer and Bligh method are;

Table 3.1: Levels of Lipids in different ages of Kei-apple fruits

Sample	Chloroform volume (ml)	Triplicate masses of precipitate (g)	Average masses of precipitate (g)	% lipids in samples
Young Kei-apples	2.6 ±0.01	0.12, 0.13, 0.15	0.133 ± 0.017	17.852±0.0010
Medium-aged Kei apples	2.3±0.01	0.08, 0.11, 0.09	0.093 ± 0.017	12.483±0.0011
Old Kei apples	3.5±0.01	0.09, 0.08, 0.07	0.08 ± 0.010	10.738±0.0014

The lipid values were found to decrease with age with significance difference of the values at 95% confidence level, (n=14, r^2= 0.9999 and Coefficient of Variation 0.00024).

Figure 6: Variation of Total lipid content of fruits with age



From above data, it was seen that the Total lipids of Kei apple fruits decreased with age. This can be attributed to lipid oxidation whereby free radicals take electrons from lipids in cell membrane resulting in cell damage. Lipid oxidation is due to both plant stress and aging. It accumulates slowly leading to reduction in lipids over time [14]. Lipid oxidation is accelerated by high temperature and humidity levels [15]. Aging is also related to an increase in oxidative products resulting from nucleic acids, sugars and other sterols [16]. There are basically two oxidation reactions i.e. auto-oxidation and photo-oxidation.

3.4 Biomineral Analysis

Iron levels were highest amongst the samples followed by zinc, cobalt and copper. Together with silicon and aluminium, iron is one of the most abundant elements on the earth [17]. The availability of the three elements above in plants however differs because they can either be beneficial (silicon), toxic (aluminium) or essential to plants. Although abundant in most aerated soils, iron abundance in plants is lower because its biological activity in plants forms highly insoluble complexes at neutral pH [18]. Abundance of copper ions in plants is not only dependent on its availability on the soil but also other factors such as pH, organic matter, dissolved Organic Carbon and Electrical Conductivity [19]. Zinc availability in plants is limited to soils above pH 6.5 above which zinc solubility decrease reducing its uptake and translocation within plants. High phosphorus levels also minimize zinc concentrations. The biosorption of cobalt ions in plants is purely species

dependent though other metals such as manganese can reduce its uptake [20].

Table 5: Concentration of selected bio-minerals in fruit samples and the nearby soil sample.

Samples	Concentration of bio minerals in ppm			
	Copper	Iron	Zinc	Cobalt
GreenKei-apples	0.01430 ± 0.000007	1.04900 ± 0.000005	0.16384 ± 0.000002	0.05604 ± 0.000005
Yellow Kei-apples	-0.01300 ± 0.000003	1.05590 ± 0.000006	0.33662 ± 0.000008	0.80190 ± 0.000004
Brown Kei-apples	0.01430 ± 0.000004	1.15780 ± 0.000001	0.21523 ± 0.000009	0.03199 ± 0.000006
Adjacent soil sample	0.01945 ± 0.000027	2.35970 ± 0.000112	0.68761 ± 0.000029	1.24612 ± 0.000117

From above data, copper ions were higher in the young and old fruits but lower in the medium aged ones, (Significantly different at 95% confidence level, n=14). Though quite immobile, copper ions slowly translocate together with other minerals from old organs to newer ones. This phenomenon is however more common in chloroplasts [21]. Its mobility is also dependent on its content in a plant. It is thus feasible to conclude that since there was less copper concentration, the mobility of copper ions in Kei apple fruits is minimized. Iron levels in the samples increased with fruit age implying continuous accumulation of the element with time. Together with copper and zinc, iron has a high density which limits its movement within plants. Therefore, iron movement and distribution between plant organs is very slow [21]. The concentration of zinc ions were highest in the medium aged fruits. Zinc uptake from soil to roots and eventually other plant organs is inhibited by some metals especially iron and manganese. Its mobility between the organs is as well very limited especially in old organs. This is attributed to its reaction with phosphorus [22]which have an antagonistic effect with each other [23]. The concentration of cobalt in plants is dependent on its interaction with other metals present in the organs as they have an almost similar biosorption means [20]. The old Kei-apple fruits indicated lower concentration of cobalt which concurs with previous findings that concentration of cobalt ions decrease with plants age [20].

3.5 Antioxidant Analysis

The relatively stable DPPH radical scavenging capacity allows it to be used to test the ability of a compound to act as a free radical scavenger or a hydrogen donor, and thus to evaluate its antioxidant activity [24]. Here, the DPPH antioxidant activities of the young Kei-apples, medium-aged Kei-apples and old Kei-apples were used to determine the antioxidant concentrations in the test sample. The half-maximum Inhibitory Concentration (IC_{50}) values were used for comparison.

Table 6: Levels of Antioxidants in different fruit ages of Kei apple fruits.

Test samples	Absorbance at 515 nm	IC_{50} ± SD (µg/mL)
Blank	0.0492 ± 0.00413	-
Young Kei-apples	0.0349 ± 0.00228	28.1385 ± 3.2224
Medium-aged Kei-apples	0.0604 ± 0.00147	-18.3985 ± 2.0187
Old Kei-apples	0.2019 ± 0.00212	-167.3160 ± 12.3030

Only the green (young) fruit samples had a considerable positive response to DPPH radical scavenging. An IC_{50} concentration of 28.1385 µg/mL was ultimately obtained. Antioxidant concentration was negatively correlated, (r^2=1, Correlation of Variance= -0.0001267) with age of the Kei-apple fruits with IC_{50} concentrations drastically decreasing from young<medium-aged<old samples. Oxidative stress due to heat and sunlight is known to decrease the bioactivity of antioxidant compounds [25, 26]explaining the linear decrease in antioxidant concentration with age of Kei-apple fruits. Only the young samples showed a significant Absorbance at 515nm corresponding to phenolic compounds in contrast to flavonoids or tannins which absorb at wavelengths close to 700nm [27]. There is however no clear elucidation that the antioxidants present in the fruit samples were wholly due to phenolic compounds and not due to other molecules such as Ascorbic Acid, Flavanoids, Flavanols, Tannins or Anthocyanins which also have antioxidant property [28].

IV. CONCLUSION

A significant difference with average f value =$6.916*10^{-6}$ and Root Mean Square of 0.0067 in nutritional composition for carbohydrates, lipids, proteins, selected bio minerals and antioxidants with age of Kei apple fruits was obtained (using 14 degrees of freedom at 95% confidence level). Young Kei-apple fruit samples indicated presence of sucrose and fructose which gradually decreased with increasing glucose levels with age. Senescence of Nitrogen from older plant organs to newer ones led to a steady decrease in protein content over time. Lipid concentration also decreased with age due to lipid oxidation in the relatively more exposed (old) fruits. Biomineral content varied between the biomineral of interest and age. Iron was the most abundant biomineral and showed a linear increase in concentration with the fruits age due to its accumulation with time and immobility between plant organs. Copper, zinc and cobalt were evenly distributed between the fruit samples. Only Young Kei-apple fruits indicated antioxidant capacity with a linear decrease in antioxidant concentration with age exhibited; possibly due to oxidative stress as a result of heat and sunlight which then decrease the bioactivity of antioxidant compounds in the fruits over time. It can be concluded that Kei apple fruits are edible fruits of significant nutritional value but varying exponentially over time as the age of the fruits increase. Nevertheless, their nutrition value will help intensify and diversify food for an ever increasing human and animal population.

ABBREVIATIONS USED

DPPH (2,2-diphenyl, 1-picrylhydrazyl), FAO (Food and Agriculture Organization), V & F (Vegetables and Fruits), VFAs (Volatile Fatty Acids), AAS (Atomic Absorption Spectroscopy), UV-VIS (UltraViolet Visible), TS (Total Solid), FTIR (Fourier Transform InfraRed Spectroscopy).

ACKNOWLEDGEMENT

The Authors thank Maasai Mara University for providing laboratory material and funds support which boosted the research activity. Also, many appreciations goes to JASCO Co Ltd for the assistance in spectrophotometric and Kjedahls process analysis.The researchers appreciate the support receivedfrom The Centre for Innovation, New and Renewable Energy Department (CINRE) of Maasai Mara University, Kenya.Many Thanks to Prof. Patricia Forbes of University of Pretoria South Africa, for reviewing the paper under request from Mr. Bakari Chaka.

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