Food Research 8 (2) : 31 - 40 (April 2024)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

RESEARCH PAPER

Impact of ripening stages on the physicochemical and antioxidant properties of Monkey orange (*Strychnos spinosa*) fruit

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Article history:
Received: 3 February 2022
Received in revised form: 20 March 2022
Accepted: 27 September 2022
Available Online: 15 March 2024

Keywords:
Monkey orange fruit,
Ripening stages,
Physico-chemical properties,
Bioactive compounds,
Antioxidant activity

DOI:
https://doi.org/10.26656/fr.2017.8(2).070

Abstract

The Monkey orange (*Strychnos spinosa*) fruit is a tropical and subtropical African indigenous tree and its name is derived from the fact that monkeys eat the fruits. This study investigated the physicochemical and antioxidant properties of Monkey orange fruit (MOF) at different ripening stages (unripe – green colour, semi-ripe - light yellow colour and fully ripe – yellow colour). Physicochemical properties, bioactive compounds and antioxidant activity of MOF were determined. The results showed that the physical characteristics of MOF such as fruit weight and size, seed weight and pulp weight significantly decreased ($p<0.05$) with the ripening stages. However, the ripening stages did not affect the seed diameter of the MOF. A significant difference was observed in the L*, a*, b* and Chroma colour properties of MOF at different ripening stages. The Hue angle of semi and fully ripe MOF was not significantly different ($p>0.05$). Chemical characteristics of MOF such as moisture content, pH, total soluble solids and sugar to acid ratio significantly increased with the ripening stages. However, extractable solids and total titratable acid decreased. Total phenolic and flavonoid contents significantly decreased with the ripening stages varying from 0.844 to 0.356 mg GAE/g and 0.714 to 0.031 mg/g, respectively. Moreover, ascorbic acid content decreased ranging from 128.67 to 73.01 mg/100 g. The antioxidant activity of MOF significantly decreased ($p<0.05$) during the ripening stages.

1. Introduction

The Monkey orange fruit (*Strychnos spinosa*) is a fruit that is traditionally utilised for nutritional and health benefits and its pulp or juice is used as the main ingredient in different types of food products (Ngadze *et al*., 2017). The Monkey orange fruit (MOF) belongs to Loganiaceae family; about seventy-five Strychnos species have been identified in Africa and around twenty species are edible. The most frequently consumed species in Southern Africa include *Strychnos innocua, Strychnos pungens,* and *Strychnos spinosa* (Nwamba, 2006). The shell of MOF is very thick and its colour is yellow or orange while the pulp has flesh, the colour is bright yellow or brown with hard seeds embedded in it. The MOF juice is produced traditionally by manually extracting the juice by mashing the pulp with a wooden spoon. Afterwards, water is added and the mixture is boiled for a few minutes before filtration (Saka *et al*., 2007). The pulp of MOF is sometimes domestically processed into products such as jams, jelly, beverages or dried leathers (Chirwa and Akinnifesi, 2008; Hassan *et al*., 2014; Adebowale *et al*., 2016). During jam production, pectin is not added since MOF has good setting properties (Saka *et al*., 2007).

The MOF is very rich in bioactive compounds that can help prevent the growth of free radicals (Ellong *et al*., 2015). Phenolic compounds have higher antioxidant activity compared with ascorbic acid (Bouayed and Bohn, 2010). The phenolic content of MOF, radical quenching and flavonoid have been reported to be relatively comparable to that of baobab nectar as catechin equivalent (Nhukarume *et al*., 2010). The MOF has a bright orange-brown pulp, and the higher colour intensity is usually associated with the high antioxidant activity of a product (Kalt, 2005). This may suggest that MOF has a high antioxidant capacity. Bioactive compounds in MOF are categorised into different classes, but phenolic acids, flavonoids and tannins are the principal dietary bioactive compounds. Various secondary metabolites composed of flavonols, flavanols, flavanones and flavones are found in the flavonoids. For tannins, hydrolysable and condensed tannins are the main classes. Hydroxybenzoic and hydroxycinnamic

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eISSN: 2550-2166
Published by Rynnye Lyan Resources

acids are phenolics (Anantharaju *et al*., 2016). Nutritionally, the MOF is high in energy, dietary fibre, vitamin C and minerals such as iron and zinc (Saka and Msonthi, 1994; Bello *et al*., 2008).

Traditionally, MOF juice or pulp is utilised as an ingredient for maize porridge (Ngadze *et al*., 2018). The pH of MOF juice is relatively very low (around 3.5) and this suggests that the fruit has antimicrobial properties (Saka *et al.,* 2007). However, yeast and mould can spoil the juice during the storage period since they can tolerate low pH. The taste of MOF is sweet or sour and it is commonly consumed by poor households in Southern Africa, especially women and children (Amarteifio and Mosase, 2006; Ngadze *et al*., 2017). The high acidity and availability of organic acids in MOF contribute to the sour taste. The sweet taste of MOF is attributed to the composition of sugar which in turn depends on the ripening stages (Lee *et al*., 2013). However, research on the evaluation of MOF properties during different stages of ripening is very limited. Therefore, this study sought to determine the impact of ripening stages on the physicochemical and antioxidant properties of MOF.

2. Materials and methods

2.1 Materials

The MOFs used for this experiment were harvested at different ripening stages at Mukula village, Limpopo province, South Africa. During harvesting, fruits were categorised as per their maturity state and ripeness: unripe (green), semi-ripe (light yellow) and fully ripe (yellow). The unprocessed fresh MOF at different stages of ripening were used to determine the physicochemical and antioxidant properties. The woody shell of MOF was cracked manually by hand to obtain the pulp (with seeds embedded in it) and each sample was stored in a sealable plastic bag at −20°C. For analysis, the frozen MOF pulp was thawed and blended to remove the seeds from the pulp.

2.2 Physical characteristics of Monkey orange fruit

2.2.1 Fruit size

A total of ten fruits were selected randomly and measured in diameter using a digital calliper.

2.2.2 Fruit weight

An analytical balance of four decimals was used to measure the weight of MOF fruit.

2.2.3 Flesh weight and seed weight

An analytical balance with four decimals was used to measure the weight of MOF fruit from the ten selected fruits. The flesh of the fruit was separated from the seed, followed by measuring the weight of each MOF seed. The weight of the flesh of each MOF fruit was determined as the difference in weight between the whole fruit and its seed.

2.2.4 Seed diameter

A digital calliper was used to measure the diameter of each seed of the MOF fruit.

2.2.5 Colour analysis

The colour of MOF was determined using the Hunter Lab System (ColourFlex, HunterLab., Reston, VA, USA) with D65 as the light source. Four replicates were taken. The L*, a* and b* values were recorded. The L* value represents (lightness/darkness), a* (redness/ greenness) and b* (yellowness/blueness). Chroma and Hue angles were measured using equations 1 and 2:

$$Chroma = \sqrt{a^2 + b^2} \quad (1)$$

$$Hue = \tan^{-1}\left(\frac{b}{a}\right) \quad (2)$$

2.3 Chemical properties of monkey orange fruit

2.3.1 pH

A digital pH meter (BOECO, Germany: Model BT-675) was used to measure the pH of the MOF sample. The standard buffer solutions of pH 4 and 7 were used to standardise the electrode of the pH meter prior to use. About 10 g of the MOF sample was mixed with 100 mL of distilled water. Then, the mixture was continuously shaken and left for 30 mins at room temperature. A pH metre was then used to measure the pH of the mixture.

2.3.2 Total titratable acid

The Association of Official Analytical Chemists (AOAC) method (1980) was used to determine the titratable acidity of the MOF sample as % malic acid.

2.3.3 Total soluble solids content

A bench refractometer (Nieuwkoop BV: Model MA871) was used to measure the soluble solids content of MOF. The residual sample was rinsed off with distilled water after each reading.

2.3.4 Sugar to acid ratio

The sugar to acid ratio was determined using the percent of the soluble solids content of the juice (expressed as °Brix) and the acidity of the juice, and the sugar: acid ratio was calculated.

2.3.5 Moisture content

The moisture content was measured by re-weighing the MOF after drying in an oven drier at 60°C for 12 hrs using 4-5 g of sample (AOAC, 2000).

https://doi.org/10.26656/fr.2017.8(2).070

2.3.6 Extractable solids

The extract (3 mL) was put in a tray, followed by drying the sample in an oven drier at 60°C for 12 hrs to remove all moisture. The extractable solid was expressed as the percentage of extractable solid per gram of dry fruit flesh.

2.4 Bioactive compound and antioxidant activity of Monkey orange fruit

For the extraction process, 2 g of MOF sample was mixed with a 20 mL methanol sample containing 1% HCl at 60±5°C (Dal Magro *et al*., 2014). The mixture was centrifuged for 20 mins at 5000 rpm. The supernatant was collected and utilised when determining bioactive compounds and the antioxidant activity of MOF.

2.4.1 Total phenolic content

The Folin Ciocalteu method was used to measure the total phenolic content of MOF (Singleton *et al*., 1999). Subsequently, 0.5 mL of MOF extract was mixed with Folin Ciocalteu reagent in a test tube and left at laboratory temperature for 5 mins. Then, 2 mL of 7.5% sodium carbonate was added after 5 mins. The mixture was incubated with occasional shaking in the dark for 90 mins. The development of a blue colour was observed after incubation. Finally, a spectrophotometer was used to measure the absorbance of blue colour in different samples at 725 nm. The calibration curve was prepared with gallic acid solution and results were expressed in milligrams of gallic acid equivalent/100 g of sample.

2.4.2 Vitamin C (ascorbic acid)

The Dichlorophenolindophenol (DCPIP) titration test was used to measure the vitamin C of MOF. For the preparation of DCPIP solution, 0.25 g of 2.6 dichloindophenol was dissolved in 500 mL of distilled water. Afterwards, 0.21 g of sodium bicarbonate was added to the mixture and allowed to melt. Distilled water was used to dilute the mixture to a litre. About 15 mL of the MOF juice was added to a 150 mL volumetric flask, followed by 40 mL of 5% acetic acid. The mixture was shaken continuously and water was added up to the mark after 20 mins. The mixture was then titrated against the prepared standard DCPIP solution (AOAC, 2000). Results were expressed as ascorbic acid equivalents per milligram of crude juice.

2.4.3 Estimation of total flavonoid content

The total flavonoid content of MOF was measured spectrophotometrically as described by Park *et al.* (2008). Briefly, 5 mL distilled water was added to 0.3 mL of extract in a test tube and 0.15 mL of 5% (w/v) $NaNO_2$ was added. Then, 0.3 mL (10% w/v) $AlCl_3$ was added after 5 mins followed by the addition of 2 mL 1 M NaOH after 6 mins. The mixture was continuously shaken and incubated in the dark for 15 mins. A spectrophotometer was used to read the absorbance of the sample at 506 nm. Catechin standard solution was used to prepare the standard curve and results were expressed as milligrams of catechin equivalents per g of dried sample.

2.4.4 DPPH radical-scavenging activity

The method of Souza *et al*. (2012) was used to measure the DPPH assay of MOF. Briefly, 3 mL of DPPH solution was mixed with 2 mL of MOF sample in a test tube. The mixture was shaken continuously and allowed to stand for 15 mins in the dark. The absorbance of the MOF sample was read at 517 nm using a spectrophotometer.

2.4.5 Ferric reducing power assay

The ferric reducing power (FRAP) assay of MOF was measured using the method of Oyaizu (1986). About 100 µL of acidified methanol extract was added to a test tube, followed by adding methanol to modify the volume to 1 mL. Afterwards, 2.46 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide were added and the mixture was vortexed. The mixture was incubated for 20 mins at 50°C and this was followed by the addition of 2.5 mL of 10% trichloroacetic acid. The mixture was centrifuged for 10 mins at 6000 rpm. A 2.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride. The absorbance of the mixture was measured at 700 nm using a spectrophotometer.

2.5 Statistical analysis

The data were expressed as mean ± standard deviation of triplicate determinations. Analysis of variance (ANOVA) and Duncan's multiple range method were used to compare the mean values. The data was generated using the SPSS package, version 26.0 (SPSS Inc., Chicago, USA). Differences were considered significant at $p<0.05$.

3. Results and discussion

3.1 Effect of ripening on physical characteristics of fresh Monkey fruit orange

Table 1 presents the impact of ripening stages on the physical characteristics of fresh MOF. There were significant differences ($p\leq0.05$) in fruit size and weight, seed diameter and weight, pulp weight and moisture as the fruit continued to ripen. MOF had a round shape with a diameter of 8-10 cm, which resembles an orange, with

https://doi.org/10.26656/fr.2017.8(2).070

Table 1. Physical characteristics of fresh Monkey orange fruit

Characteristics	Unripe fruit	Semi ripe fruit	Fully ripe fruit
Fruit weight (g)	544.98±120.15^{c}	348.48±133.57^{b}	300.95±104.80^{a}
Fruit size (cm)	9.58±1.17^{c}	8.77±1.28^{b}	8.41±0.83^{a}
Seed weight (g)	245.24±54.07^{c}	142.88±60.11^{b}	111.35±47.16^{a}
Pulp weight (g)	272.49±60.07^{c}	188.18±66.78^{b}	174.55±52.40^{a}
Seed diameter (cm)	2.04±0.56^{a}	1.97±0.57^{a}	2.03±0.59^{a}
Fruit colour			
L*	64.99±0.02^{c}	38.21±0.01^{b}	37.25±0.03^{a}
a*	1.73±0.04^{a}	12.48±0.05^{c}	11.36±0.07^{b}
b*	42.09±0.14^{c}	30.71±0.14^{b}	27.87±0.12^{a}
Chroma	42.46±0.59^{c}	33.15±0.13^{b}	30.09±0.09^{c}
Hue angle	87.64±0.06^{b}	67.88±0.08^{a}	67.81±0.20^{a}

Values are presented as mean±SD. Values with different superscripts within the same row are statistically significantly different ($p<0.05$). FS: Fruit size, FW: Fruit weight, SD: Seed diameter, PW: Pulp weight, SW: seed weight.

a mean diameter of around 9.5 cm when not fully ripe and 8.4 cm when fully ripe. The fruit weight ranged between 300 and 600 g, unripe MOFs had a significantly ($p<0.05$) higher weight than semi-ripe and fully ripe ones, and the fruit was much heavier than most of the commercially produced subtropical and citrus fruits such as apples, kiwis, mangos and oranges. However, the value was low when compared to that of pineapples, papayas and watermelons (Contreras-Calderón *et al.,* 2011; Liu *et al*., 2012; Shukla, 2017). The low weight of MOFs may be due to the loss of water during respiration which breaks down complex materials in fruit cells into simpler molecules that give energy and certain molecules which are utilised in various cellular reactions. Moreover, the decrease in weight might also be attributed to the polysaccharides that undergo modifications such as starch, pectins, cellulose, and hemillose which in most fruits cause some textual changes and fruit softening due to depolymerisation during ripening. Unripe MOFs had a significant ($p<0.05$) higher seed weight than semi and fully ripe MOFs. Seed weight ranged between 100 and 250 g, which forms about 35% to 45% of the total fruit. Unripe MOF had a higher seed weight (245.24 g) than semi-ripe (142.88 g) and fully ripe (111.35 g). Unripe MOF had a higher pulp weight (272.49 g) than semi-ripe (188.18 g) and fully ripe (174.55 g) MOF. Pulp forms about 50% to 60% of the total fruit. Similar results were observed by Mizrahi *et al.* (2002) on the characteristics of monkey orange fruit who reported that it consists of 30% to 45% juicy flesh. At around 2 cm, the seed diameter of MOF was not significantly ($p>0.05$) affected during ripening. The ripening stages resulted in low diameter which is associated with dehydration during the ripening process and a decrease in the peel weight. The changes in the peeling quality during the ripening stages are due to the collapse of the cell wall which leads to the generation of air spaces in the middle lamella.

The results of the colour analysis of MOF are shown in Table 1. The lightness (L* values) of MOF ranged between 64.99 and 37.25. The unripe fruit was brighter than semi-ripe and fully ripe ones. The lightness value of three different stages of ripening was significantly different ($p<0.05$) as observed ripening stages reduced the lightness of MOF from light to dark brown colour. The development of dark colour might be due to enzymatic and Maillard browning reactions that generate water-soluble brown, grey, and black coloured pigments. The redness (a* values) of MOF ranged between 1.73 and 11.36. Unripe MOF was more reddish than semi-ripe and fully ripe ones. Orazem *et al.* (2011) proposed the utilisation of a* value as an appropriate maturity index since it is the main colour coordinate that changes during the ripening process. The yellowness (b* values) of MOF ranged between 27.87 and 42.09. Unripe MOF had a lower value of yellowness (42.09) than semi-ripe (30.71) and fully ripe (27.87) ones. This may be due to the compounds which contribute to the yellow pigment of MOF which is caused by the presence of carotenoids and flavonoids. The enzymes, such as polyphenol oxidase, catalyse the oxidation of polyphenolic compounds, carotenoids and flavonoids resulting in the decrease of MOF yellowness. The values for the Hue angle of MOF ranged from 87.64 to 67.81 and decreased as the MOF fully ripe and became brown in colour. With regards to the hue angle, the highest value was obtained in green fruit with a hue of 87.64, while the fully ripe MOF had a value of 67.81 (yellowish colour). These values are higher compared to those of Adriano *et al.* (2011) who found values of 39.81 for semi-ripe fruit (orange colour) and 30.08 for ripe fruit (reddish).

3.2 Influence of ripening stages on the chemical characteristics of Monkey orange fruit

The impact of ripening stages on the chemical properties of MOF is presented in Table 2. The moisture

https://doi.org/10.26656/fr.2017.8(2).070

Table 2. Chemical properties of Monkey fruit orange.

Attributes	Unripe fruit	Semi ripe fruit	Fully ripe fruit
Moisture content (%)	78.73±1.10[a]	80.25±0.50[b]	82.76±0.11[c]
Extractable solids (%)	21.26±1.10[c]	19.74±0.51[b]	17.23±0.11[a]
Total soluble solid (°Brix)	17.23±0.11[a]	17.68±0.03[b]	18.65±0.01[c]
pH	3.79±0.04[a]	3.88±0.01[b]	3.96±0.01[c]
Total titratable acidity (%)	1.51±0.02[c]	1.24±0.03[b]	1.13±0.02[a]
Sugar to acid ratio	10.90±0.17[a]	14.16±0.32[b]	16.42±0.40[c]

Values are presented as mean±SD. Values with different superscripts within the same row are statistically significantly different ($p<0.05$).

content of MOF showed a significant increase ($p<0.05$) during the three ripening stages. The increase in the moisture content of the fruits during maturation was likely caused by the moisture loss from the pulp to the peel. Carbohydrates are hydrolysed into sugar during maturation and increase osmotic moisture conversion from peel to pulp (Adeyemi and Oladiji, 2009). Therefore, it was expected that the moisture content of the MOF pulp would increase. Water is an essential solvent for many soluble substances including salts and sugars which are known to affect fruit flavour. Increased humidity in fruit pulps during maturation could thus enhance the taste and aroma of the fruits. It accounts for the softening appearance of MOF fruit as it matures. Bhatnagar *et al*. (2019) and Ahenkora *et al*. (1997) indicated that water vapour pressure in fruits and their atmosphere triggers a moisture depletion process in fresh fruits and vegetables. The loss of fruit and vegetable humidity is related to the disintegration of cell membranes contributing to cellular material leakages to exacerbate senescence.

During maturation, extractable solids significantly decreased ($p<0.05$) to between 17% and 22%, whereas unripe MOF had the highest value (21.23%) and fully ripe had the lowest value (17.21%). The low moisture content and the hydrolysis of the material of starch, organic acids, and phenols (tannins), which are components of extractable base, may be important, whereas the amount of nitrogen compounds and soluble sugars decreases during maturation of the fruit (Borsani *et al*., 2009). The degradation of pectin into soluble by-products may be due to the maturation (Tapre and Jain, 2014; Kumar *et al*., 2015). Similar results were obtained by Marin and Cano (1992) in the analysis of peroxidase variations in the ripening of mango (*Mangifera indica*, L.).

The total soluble solids (TSS) values of the ripening stages of MOF ranged from 16.52 to 18.65. All three ripening stages showed significant differences at $p<0.05$. The TSS continuously increased during the ripening process of MOF. The lowest value (16.52) was observed during the unripe stage and the highest value (18.65) during the fully ripe stage of MOF. Magwaza and Opara (2015) have shown that TSS is a measure of quality that demonstrates how sweet, fresh and processed foods are. Studies with different types of fruits reported increases in TSS during the ripening period (Wu *et al*., 2005). Jiménez *et al*. (2011) showed that the TSS of Gulupa (*Passiflora edulis*) fruit increased during ripening ranging from 13.5 to 17.4%. Sitrit *et al*. (2003) reported a sugar and organic acid accumulation during the ripening process and conversion of sucrose into glucose and fructose at the start of MOF ripening. Saccharose, glucose and fructose are the main sugars that accumulate during ripening. The low TSS during the early storage period is caused by the fact that starch would not have been enzymatically degraded and converted into sugar. The gross soluble pulp solids rise with the decrease of the accumulated starch in the pulp to sugars (Dadzie and Orchard, 1997; Zhang *et al*., 2005; Seymour *et al*., 2012). The TSS is therefore related to the stages of fruit maturation.

The pH of the ripening stages of MOF ranged from 3.79 to 3.96. Out of the three stages, unripe MOF had the lowest pH (3.79) while the fully ripe one had the highest pH value (3.96). During the ripening process of MOF, pH increased while the acidity decreased. The same pattern was observed by Adriano *et al*. (2011) who found higher pH values in fully ripe than semi-mature fruits, but the opposite happens to titratable acidity. Butt (1980) states that enzyme production in full-matured fruits shows greater activity than in unripe fruits by enzymes such as oxidase ascorbic acid (ascorbate oxidase), which means that the overall acidity of the MOF is decreased and pH increases when it matures. Such principles come within the framework of this current study. At the first level of development, the elevated pH, which declined during the maturation process, is likely to be due to a rise in fruit organic acid content (Arvanitoyannis and Mavromatis, 2009). The relatively high acidity of MOF can contribute to fresh fruit's shelf life compared to other fruits. Oliveira *et al*. (1999) obtained pH values between 2.50 and 3.30, i.e. greater than in the current study, with frozen acerola pulp.

The titratable acids of unripe fruit had a high value (1.51) than semi-ripe (1.24) and fully ripe fruits (1.13). A

https://doi.org/10.26656/fr.2017.8(2).070

decrease in the titratable acidity of fruits during ripening stages has been reported (Rooban *et al*., 2016). Titrable acidity measures the amount of acid in fruit. The decrease in acidity could be attributed to citric acid susceptibility to oxidative degradation because of ripening conditions (Abacı and Asma, 2013). The acidity decreases suggest softening with the potential to improve the sweet taste as shown in MOF. Gunduz *et al*. (2013) suggested that low acidity during maturation is due to the increase of total sugars and a drop of acidity arising from starch hydrolysis. The low titratable acidity of MOF might also be due to the low concentration of organic acids during the ripening process as the accumulation of soluble solids increases because of easy dilution and the use of acids in the plant respiratory process (Prinsi *et al*., 2011). Acidity during fruit maturation is commonly observed and is caused by the deposition of organic acids. Many organic acids are used during maturity as substrates for respiratory activity and therefore reduce titrable acidity. The results of this study are comparable to those reported by Cordenunsi *et al*. (2002). Similar results of 0.47 to 1.56% in citric acid were also obtained in acerola pulp (Mercali *et al*. 2012).

The ratio of sugar to the acid of MOF varied from 10.90 to 16.42. During the ripening process, there was an increase in the ratio; fully ripe MOF had the highest value (16.42), while unripe MOF had the lowest value of 10.90. The ratio is an important quality feature for fruits as it determines the taste and choice of raw materials. Thus, this association tends to grow during maturation. Adriano *et al*. (2011) found the same trend but reported a lower value of 2.41 for mature fruits. Low total acidity and high total sugars are principal factors for flavour development. The sugar/acid ratio is used to measure fruit maturity and increases with fruit maturation and decreases with fruit senescence (Magwaza and Opara, 2015; Makumbele *et al*., 2019). Similar results in cherry fruits were also reported by Gunduz *et al*. (2013).

3.3 Effect of ripening on the bioactive compounds and antioxidant activity of Monkey orange fruit

Table 3 shows the effect of ripening stages on the bioactive compounds and antioxidant activity of MOF. The total phenolic content showed significant variation ($p<0.05$) in all three stages of maturation. Phenolic compounds are well-known to contribute to fruit consistency and nutritional worth through the modification of colour, appearance, flavour and beneficial health effects. The highest phenolic content was observed in unripe MOF (0.844 mg GAE/g) and the least was in fully ripe MOF (0.356 mg GAE/g). The decrease in total phenolic content can be attributed to intervening components in the juice pulp, including sugar and amino acids that can interfere with the Folin Ciocalteu reagent. Moreover, environmental factors such as sunlight and temperature contribute to a decrease in phenolic content during sample analysis since phenolic compounds are very sensitive to these two factors. By contrast, Siriwoharn *et al*. (2004) found no significant improvements in the quality of complete blackberry mature phenols. The total phenolic content in strawberries has been shown to remain almost stable from white to purple-red fruit (Ferreyra *et al*., 2007). The total flavonoid content of MOF significantly decreased ranging from 0.174 to 0.031 mg/g, wherein unripe fruits had the highest value and fully ripe one the lowest value. The enzyme (polyphenol oxidase) is involved in browning reactions during the ripening of MOF oxidised polyphenolic compounds, carotenoids and flavonoids which resulted in the decrease of total flavonoid content. The decrease in flavonoid content may be due to the maturity of the fruit which is followed by accelerated metabolism, particularly in climacteric fruit and this leads to solubilisation and biochemical breakdown of pectin (Wang and Lin, 2003). Similar findings were obtained by Silva *et al*. (2019) who observed that the flavonoid and flavonol contents decreased with the ripening stages in apple fruit.

With regards to the ascorbic acid, all three ripening stages showed significant differences at $p<0.05$. Fully ripe MOF presented an ascorbic value of 73 mg/100 g, hence lower than fully green MOF (128.67 mg/100 g). In other words, the more the fruits ripened, the lesser the ascorbic acid content. Lower ascorbic acid occurs because an enzyme oxidase ascorbic acid (ascorbate oxidase) presents higher activity in fully ripe fruits than in unripe fruits. This explains some of the losses found during the ripening stages (Butt, 1980). Zielinski *et al.* (2014), observe that unripe fruits exhibit ascorbic acid levels significantly higher than mature fruit and semi-

Table 3. Bioactive compounds and antioxidant activity of Monkey fruit orange.

Attributes	Unripe fruit	Semi ripe fruit	Fully ripe fruit
TPC (mg GAE/g)	0.844 ± 1.10^{c}	0.565 ± 0.50^{b}	0.356 ± 0.11^{a}
TFC (mg CE/g)	0.174 ± 1.10^{c}	0.126 ± 0.51^{b}	0.031 ± 0.11^{a}
Ascorbic acid (mg/g)	128.67 ± 0.11^{c}	92.40 ± 0.03^{b}	73.00 ± 0.01^{a}
DPPH (%)	70.14 ± 1.22^{c}	61.08 ± 0.47^{b}	50.32 ± 0.82^{a}
FRAP (mg GAE/g)	3.21 ± 0.01^{c}	2.45 ± 0.04^{b}	1.40 ± 0.01^{a}

Values are presented as mean±SD. Values with different superscripts within the same row are statistically significantly different ($p<0.05$).

https://doi.org/10.26656/fr.2017.8(2).070

mature, consequently, being used by the pharmaceutical industry. In the current study, the ascorbic acid level (17.23 mg/100 g of fresh weight) in fully ripe MOF is lower than that recommended by FAO/WHO (2001), i.e. 45 mg per day. Similar results were observed by Pineli *et al*. (2011) on two strawberry cultivars at different ripening stages.

The DPPH scavenging activity value of unripe MOF (70.14%) was significantly higher ($p<0.05$) than that of semi-ripe (61.08%) and fully ripe ones (50.32%) MOF. Similar results were reported by Gunduz *et al*. (2013) wherein the antioxidant activity of cornelian cherry fruits decreased with the maturation. The presence of phenolic acid, flavonoids and ascorbic acid contributes to the antioxidant activity of the fruit. Maturity had an important influence on experimental antioxidant activity (DPPH). Throughout maturation, a progressively decreasing pattern of FRAP activity was observed and the results were in accordance with DPPH. The FRAP values of MOF varied from 3.21 to 1.40 with fully ripe fruits having a low value. During fruit maturation, organic acids decrease since most acids are used during the respiration process. Moreover, during fruit ripening, most acids are changed into sugars, hence the reduction in FRAP (Gunduz *et al*. 2013). The results are consistent with Liu *et al*. (2019) wherein FRAP values significantly decreased during the ripening stages of five peach cultivars.

4. Conclusion

The results of this study show that ripening stages influence the physicochemical and antioxidant properties of MOF. Significant variations were observed whereby physical characteristics decreased with the ripening stages. In terms of the colour, the a* value increased with the ripening stages while other parameters such as L*, b*, Chroma and Hue angle decreased. Chemical properties with different amounts were recorded in all three ripening stages of MOF and significantly varied. High bioactive compounds and antioxidant activity were recorded in unripe MOF. The MOF can be used in food products as a functional ingredient since it is rich in ascorbic acid at the maturity stage. More studies on the quantification of polyphenolic compounds of MOF during various ripening stages should be conducted.

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