



African Star Apple Juice Stored at Tropical Ambient and Refrigeration Temperatures: Effect on Physicochemical Characteristics, Antioxidant Properties and Microbiological Quality

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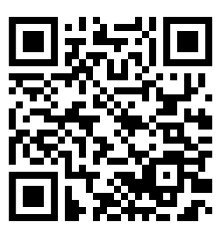

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Abstract	Article History
<p>The present study was aimed at evaluating the physicochemical and antioxidant properties as well as microbiological quality of extracted juice from African star apple (<i>Chrysophyllum albidum</i>) stored under tropical ambient and refrigeration conditions. Juice was extracted from mashed star apple fruit cotyledons (after deseeding) and pasteurized in a water bath at 71.2 °C for 30 minutes before storage for four (4) weeks under tropical ambient (28±2 °C) and refrigeration (6±2 °C) conditions. The results showed that there was no fibre in the juice, the moisture was 78.51% and 79.13% for fresh African star apple (ASA) juice and sample stored under ambient conditions, respectively. It also showed pH of ASA juice at week 0 to be 3.52 and week 4 for both samples stored at refrigeration and ambient temperatures to be 2.85 and 2.28, respectively. There was no fecal coliform count in all the studied juice samples throughout the storage period, but total viable bacteria counts were 2.7x10⁴ cfu/ml, 1.84x10⁵ cfu/ml and 6.6x10⁴ cfu/ml for fresh juice and juice stored for 4 weeks at ambient temperature and refrigeration conditions, respectively. Overall, storage conditions had insignificant impact on the antioxidant properties and free radical scavenging capacity of the fruit juice; the slight changes observed were linked to usual molecular reactions. In conclusion, storage under refrigeration conditions elongated the shelf life of the juice.</p> <p>Keywords: African star apple; stored juice; nutritional quality; microbial counts; antioxidants</p>	<p>Received: 21 Feb 2024 Accepted: 02 Mar 2024 Published: 08 Mar 2024</p> <div style="text-align: center;">  Scan QR code to view* License: CC BY 4.0*  Open Access article. </div>
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1. Introduction

In the developing countries, more attention is paid to value-addition and healthy-nutrition promotion when developing any food product (Enujiugha, 2000; Talabi *et al.*, 2023). In this regard, tropical fruits and commonly available vegetables are among the most important foods, as they are not only nutritive but are also indispensable for the maintenance of health (Dabesor *et al.*, 2022; Wong *et al.*, 2003), marking them out as functional ingredients in food preparations. They also contribute to dietary diversity, especially in countries that rely heavily on monotonous starchy meals and porridges (Enujiugha, 2020). Fruits and vegetables are noticeably abundant during their various seasons of glut, with over 50% lost to wastage owing mainly to deterioration under tropical conditions due to high ambient temperatures and humidities, pest and diseases infestation, poor handling and storage

facilities (Dauda *et al.*, 2017). Biodeterioration of tropical fruit and their products is influenced by factors like temperature, pH, chemical composition and microbial load.

African star apple (*Chrysophyllum albidum*) is an unconventional and wild forest fruit tree that is commonly scattered throughout tropical Africa. *Chrysophyllum albidum* fruits contain a high nutritional value, and are rich mainly in vitamin C, thereby making a good nutritional option in relation to the quality attributes and flavor (Oguntimehin *et al.*, 2022a,b). The fleshly pulp of the fruit is widely consumed by the diverse local populations and it can taste either very sweet or sour. Indigenously, the variation of the fruit exocarp color is said to be correlated to the pulp taste. The exocarps of the sweet varieties are yellow while those of the sour accessions have a mixture of yellow and green colours when matured.

Previous studies on *C. albidum* in western Africa reported on the importance of the species for local community livelihood improvements and its potentiality for utilization in the food industries (Oguntimehin *et al.*, 2021), as well as the effect on antioxidant enzymes and polyphenol oxidase activity (Bobadoye *et al.*, 2016; Enujiugha *et al.*, 2023).

Nutritionally, African star apple pulp contains higher vitamin C content at approximately 446 mg/100 g when compared to mango, pineapple, pawpaw and hog plum at 98.0, 38.3, 39.3 and 10.1 mg ascorbic acid per 100 g, respectively (Edem *et al.*, 1984; Ellong *et al.*, 2015; Stadlmayr *et al.*, 2010; 2012)). The bright orange colour of the fruit darkens as it gets ripen therefore it is advised to go for a dark brown coloured African star apple to get a sweeter and less tart taste. Ripe African star apple confers a complex taste experience, depending on the level of maturity and ripening stage, ranging from sour to amazingly sweet. However, irrespective of the vast consumption of this fruit and its significant contribution to the nutritional intake of Nigerians, its seasonality limits its availability throughout the year coupled with losses that take place shortly after harvesting and deterioration problems during storage or when processed largely due to the microbial and biochemical changes associate with tropical environments. The fruit pulp is rich in vitamin C and iron and an excellent source of raw material for industries (Akubugwo and Ugbogu, 2007). Studies have shown a diminished risk of chronic diseases in populations consuming diets high in fruits and vegetables and it has been suggested that antioxidants found in large quantities in fruits and vegetables (Enujiugha *et al.*, 2014) may be responsible for this protective effect. This study therefore examined variations in some parameters that define the quality of African star apple juice, as influenced by storage under tropical ambient and refrigeration conditions. This was with a view to establishing minimum storage requirements for this highly functional fruit product.

2. Materials and Methods

Sample Collection

Fresh fruits of African star apple (*Chrysophyllum albidum*) were obtained from a local farm in Owena, Osun state. Plastic bottles and muslin cloth used in the study were procured from the main market (Oja Oba) in Akure, Ondo state. All the chemicals and reagents in the study used were of analytical grade, and procured from a certified laboratory materials supplier in Akure.

Production of African Star Apple Juice

Juice was extracted from the African star apple fruit using a previously described method (Enujiugha *et al.*, 2023). Briefly, the fruits were first sorted and washed thoroughly with clean water to remove any adhering substances, subsequently peeled and its seeds were removed. The fleshy cotyledon was sliced into small pieces using sharp stainless-steel knife and blended until it became semi-solid mass. A mesh cloth (muslin) was used to remove solid materials from the juice. The juice extracted was then filled into sterilized glass bottles and then pasteurized in a water bath at 71.2 °C for 30 min. The juice was divided into two equal parts. The first part was stored under ambient temperature (28±2 °C), while the other was

stored at refrigerated temperature (6±2 °C), and employed for further analysis at the end of the storage period.

Determination of proximate chemical composition

The proximate chemical composition of the fresh and stored African star apple juice samples was carried out using the methods outlined by AOAC (2012). Moisture content was determined by the air oven method at 105 °C until constant weight is reached. Fat content was estimated using Soxhlet extraction apparatus via 6 hours of n-hexane-enhanced exhaustive extraction, at the end of which the solvent was evaporated off. Crude fibre was estimated after digesting a known weight of fat-free sample in a mixture of refluxing 1.25% sulphuric acid and 1.25% sodium hydroxide, with subsequent oven drying (105 °C for 2 h) and ashing (500 °C for 4 h). Ash content was carried out in a Muffle furnace at 550 °C for 8 h to burn off all organic residues (Asunni *et al.*, 2024). Crude protein was by the semi micro-Kjeldahl technique. Carbohydrate content was estimated via the difference method (subtracting the percent crude protein, crude fibre, crude fat, and ash from 100% dry matter).

Determination of Physicochemical Properties of African Star Apple Juice

The pH of the juice samples was measured using digital pH meter (ELICO L1 614 pH analyser) and the values were expressed in pH units. The total titratable acidity (TTA) was determined by titrating a known sample with 0.01 M NaOH using phenolphthalein indicator (Badejo *et al.*, 2017). Briefly, a known volume (10 ml) of the juice sample was pipetted into a beaker and 1 drop of phenolphthalein was added. The mixture was then titrated against the standard 0.01 M sodium hydroxide solution until a light pink colour was attained. The reading of the burette was recorded and used in calculating %TTA. The total soluble solids (reducing sugars) content was determined using a hand-held refractometer model RX 1000 (Atago Co. Ltd., Tokyo, Japan) and recorded as °Brix (Ojo *et al.*, 2017).

Vitamin C (ascorbic acid) content of the African star apple juice was determined using the method previously described by Bobadoye *et al.* (2016). Briefly, 75 µl DNPH (2 g dinitrophenyl hydrazine, 230 mg thiourea and 270 mg CuSO₄·5H₂O in 100 ml of 5 mol·l⁻¹ H₂SO₄) was added to 200 µl reaction mixture (300 µl of an appropriate dilution of the polar extract with 100 µl 13.3% TCA and water). The reaction mixtures were subsequently incubated for 3 hours at 37 °C, then 0.5 ml of 65% H₂SO₄ (v/v) was added to the medium, and the absorbance was measured at 520 nm. The vitamin C content of the juice was subsequently calculated from the absorbance values.

Analysis of Phytochemicals in African Star Apple Juice

The determination of the total flavonoid content (TFC) of samples was by the Aluminium chloride (AlCl₃) colorimetric method. Briefly, 1.5 ml of each sample was mixed with 5 ml distilled H₂O and 0.3 ml of 5% NaNO₂; then 1.5 ml of 2% methanolic AlCl₃ solution was added after 5 minutes. Double distilled water (ddH₂O) was used instead of sample as blank. Two millilitres of 1 mol/L NaOH was added after 5 min and volume made up to 10 ml with ddH₂O. Mixture was shaken on orbital shaker for 5 min at 200 rpm. Absorbance was taken at

367 nm after 10 min incubation period. Total flavonoid content was calculated using a standard calibration curve prepared for quercetin and expressed as mg quercetin/100 mL of sample (Enujiugha *et al.*, 2014).

The Folin–Ciocalteu method was used to quantify the total phenolic compounds by spectrophotometry. Briefly, 0.5 ml of sample was introduced into test tubes, followed by the addition of 2.5 ml of 10% Folin Ciocalteu reagent and 2 ml of 7.5% Na₂CO₃. Mixture was allowed to stand for 30 min at 37 °C and absorbance was read at 765 nm. Total phenolic content was expressed as milligram of gallic acid equivalent (GAE) per ml of sample (mg GAE/mL) (Enujiugha, 2010).

Phytate analysis was carried out following a modified procedure of Latta and Eskin (1980). Sample (2.0 ml) was extracted with 40 ml of 2.4% HCl (68.6 ml of 35% hydrochloric acid in total volume of 1 L of H₂O) while constantly shaking at 25 °C for 3 h. All extracts were then filtered and phytate content determined at 640 nm in a spectrophotometer. Amount of phytic acid was calculated from organic phosphorus (Enujiugha and Olagundoye, 2001). For the determination of tannins, a known volume (2.0 ml) of the sample was weighed into each 50-ml sample bottle. Then 10 ml of 70% aqueous acetone was added and the bottle was properly covered. The bottles were put in an ice bath shaker and shaken for 2 hours at 30 °C. Each solution was then centrifuged and the supernatant stored in ice. About 0.2 ml solution was pipetted into the test tube and 0.8 ml of distilled water was added. Standard tannic acid solutions were prepared from 0.5 mg/ml of the stock and the solution made up to 1 ml with distilled water. Folin Ciocalteu reagent (0.5 ml) was added to both sample and standard, followed by 2.5 ml of 20% Na₂CO₃ and the solutions were then vortexed and allowed to incubate for 40 min at room temperature. The absorbance was read at 725 nm against a reagent blank concentration of the same solution prepared based on a standard tannic acid curve (Makkar and Goodchild, 1996).

Microbial Analysis

Diluent (physiological saline solution) and respective media (Nutrient Agar, NA for bacteriological analysis and Potato Dextrose Agar, PDA for fungal analysis) were prepared according to manufacturers' specifications for standard microbiological enumeration (Babatuyi *et al.*, 2019). The juice was serially diluted and plated from 10⁻², 10⁻³, 10⁻⁴ and 10⁻⁵ dilutions using spread plate method. After incubation, all colonies were counted and recorded as colony forming units (cfu) per ml.

Sensory Evaluation

Organoleptic properties of African star apple juice samples were evaluated by 30 untrained panelists, for various sensory attributes (colour, taste, aroma, mouthfeel, overall acceptability) using a 9-point Hedonic scale (Makanjuola and Enujiugha, 2015), where 9 represented "extremely like" and 1 represented "extremely dislike." All the panelists were presented fresh samples after rinsing their mouths with portable water in-between tested samples.

Statistical Analysis

The experimental design used was completely randomized design, and data collected were analyzed using one-way analysis of variance. Means were separated by Duncan's new multiple range test, and the level of significance was accepted at (p<0.05).

3. Results and Discussion

Proximate Composition of African Star Apple Juice during Storage

The moisture content of any food is usually taken as a measure of its water activity and is generally used as an index of stability and susceptibility to microbial contamination. Moisture contents of the samples in this study were very high (Table 1). This is in agreement with the reports of Ekanem and Ekanem (2018) and Hashimi *et al.* (2007) which recorded very high moisture contents in various species of apple. The high content of moisture in the samples suggests that they have high perishability (Adeleke and Abiodun, 2010). This implies that ASA fruit juice may have a short shelf life due to its high moisture content. For both storage temperatures, variations were observed to occur. According to Shahnawz *et al.* (2012), storage temperature affects the moisture content of fruits during storage.

The protein contents of the stored ASA juice at both ambient and refrigerated temperatures were observed to decrease with increase in the storage period. However, the refrigerated juice was able to retain more protein than the juice stored at ambient temperature. This may be as a result of the low temperature employed which reduced the rate of microbial degradation, since the organisms tend to use the protein as feed stock for survival and growth. This is similar to the findings of Ndife *et al.* (2014) on stored soursop juice

In the food matrix, fat is an excellent source of energy, enhances transport of fat soluble vitamins, protects internal tissues and contributes to important cell processes. The fat contents of the stored juice in this study were observed to decrease as the storage period increased with more decrease observed in the juice stored at refrigerated temperature which may be as a result of the cold conditions in the storage environment. Oguntimehin *et al.* (2021) also observed similar results and reported slight changes in proximate chemical composition during tropical ambient storage. For this particular fruit, the fat content is usually not an important factor in its shelf life determination, as the content is not quite significant to create major degradation reactions.

The amount of ash present in a food sample can be translated to the quantity or concentration of minerals present (Coimbra and Jorge, 2011). The ash content of the juice stored at both ambient and refrigerated storage decreased with prolonged storage period, although not too significant for juice stored at refrigerated temperature. However, there was significant difference in the ash content of the freshly prepared juice and the one stored at ambient temperature at the end of the storage period. Ash is the inorganic residue remaining after the water and organic matter are usually removed (Shahnawz *et al.*, 2012), and in this study it was observed to change slightly.

Carbohydrate is an essential nutrient in the body as it is the major energy source (Garuba *et al.*, 2018). Variations were observed to occur in the carbohydrate content of the stored ASA juice. However at the end of the storage period, the juice stored at refrigerated temperature was observed to contain more carbohydrates and sugars compared to the juice stored at

ambient temperature. These variations could be as a result of the different reactions associated with increased temperatures. The low refrigeration temperature helped to inhibit some reactions that could have resulted in spoilage and quality depreciation in the juice.

Table 1: Proximate Composition (%) of African Star Apple Juice during Storage

Storage Time (Weeks)	Sample	Moisture (%)	Protein (%)	Fats (%)	Carbohydrate (%)	Ash (%)
0	ABC	78.51±0.72 ^a	4.39±0.09 ^d	1.99±0.01 ^c	14.29±0.51 ^g	0.83±0.01 ^c
1	AFE	85.49±0.12 ^f	4.07±0.02 ^c	1.90±0.05 ^{bc}	2.33±0.06 ^a	0.77±0.01 ^{bc}
	RDE	84.27±0.04 ^e	4.03±0.04 ^c	1.98±0.04 ^c	8.88±0.01 ^c	0.86±0.04 ^c
2	AFE	82.69±0.06 ^d	3.71±0.09 ^b	1.97±0.05 ^{bc}	11.62±0.05 ^e	0.76±0.04 ^{bc}
	RDE	80.78±0.28 ^c	3.57±0.28 ^b	1.96±0.08 ^{bc}	12.91±0.07 ^f	0.79±0.01 ^{bc}
3	AFE	87.05±0.07 ^h	2.26±0.01 ^a	1.92±0.03 ^{bc}	8.08±0.09 ^b	0.69±0.03 ^a
	RDE	84.59±0.05 ^e	2.25±0.04 ^a	1.83±0.01 ^a	10.53±0.02 ^d	0.77±0.02 ^{bc}
4	AFE	86.58±0.03 ^g	2.20±0.03 ^a	1.86±0.06 ^b	8.84±0.04 ^c	0.61±0.03 ^a
	RDE	79.13±0.11 ^b	2.25±0.04 ^a	1.84±0.01 ^a	16.04±0.01 ^h	0.75±0.04 ^{bc}

Mean (±SEM) with different alphabetical subscripts in the same column are significantly different at $p < 0.05$.

ABC- Refreshly extracted African Star apple Juice; AFE -African Star apple juice stored at ambient temperature; RDE - African Star apple juice stored at refrigeration temperature

Physicochemical Properties of African Star Apple Juice during Storage

The pH of the fruit juice at ambient and refrigerated temperature storage decreased as the storage time increased (Table 2). The decrease observed in the pH could be related to the action of the citric acid leading to slight degradation of its sugar content and this might be because of the microbial growth that produced lactic acid. Most bacteria will not grow at low pH, which could have contributed to the shelf stability of the juice at the early stages of storage, and thus maintaining good keeping quality (Dauda *et al.*, 2017).

Total titratable acidity of the sample stored under ambient temperature increased as the storage period increased. Also, for the refrigerated storage, it increased till the end of the second week, drastically reduced by the third week, and later experienced slight increase at the 4th week of storage. The increase observed might be as a result of the stable concentration of the organic acid in the juice. This was similar to the findings of Dauda *et al.* (2017). According to Khajehei *et al.* (2015), as TSS increased, an increase in the organic acid content was observed, affecting the pH and TTA values. The findings in this study were similar those obtained by Orellana-

Palma (2020) in pineapple and apple juice and blueberry juice, in which all the juices had antagonistic values in pH and TTA with the increase in solutes as the storage period increased.

Total soluble solids, indicated by the °Brix values, of the sample kept under ambient temperature decreased as the storage period increased as presented in Table 2. The gradual reduction noticed in the values of TSS might be due to the utilization of sugars by fermenting organisms, which could have led to the gradual degradation noticed (Lemos *et al.*, 2020). This agreed with the report of Dauda *et al.* (2017) for juice from African star apple fruits stored after processing. Also, the results were comparable to the findings of Wahia *et al.*, (2020) who studied melon juice and their quality properties preservation at various days during storage. For storage at refrigerated temperature, the total soluble solid gradually increased till the end of the 4th week. This increase in total soluble solids under refrigerated storage might be due to low temperature, thus reducing hydrolysis of poly-saccharides and acids. Similar results were also reported by Bhardwaj and Nandal (2014) in Kinnow Mandarin juice blends, Prasad and Mali (2010) in Kinnow juice.

Table 2: Physicochemical Properties of African Star Apple Juice during Storage

Storage Time (weeks)	Sample	pH	TTA (%)	°Brix
WEEK 0	ABC	3.52±0.03 ^g	9.68±0.05 ^a	16.71±0.13 ^e
WEEK 1	AFE	3.39±0.09 ^f	10.96±0.06 ^c	14.26±0.06 ^d
	RDE	3.45±0.05 ^{fg}	10.55±0.04 ^b	18.99±0.30 ^f
WEEK 2	AFE	2.99±0.01 ^d	11.54±0.04 ^e	10.96±0.08 ^c
	RDE	3.16±0.06 ^e	12.79±0.30 ^g	18.96±0.08 ^f
WEEK 3	AFE	2.55±0.04 ^b	12.34±0.04 ^f	8.99±0.15 ^b
	RDE	2.92±0.02 ^{cd}	11.26±0.02 ^d	18.99±0.15 ^f
WEEK 4	AFE	2.28±0.05 ^a	12.92±0.03 ^g	8.45±0.06 ^a
	RDE	2.85±0.01 ^c	11.64±0.03 ^e	21.42±0.03 ^g

Mean (±SEM) with different alphabetical subscripts in the same column are significantly different at $p < 0.05$.

ABC- Refreshly extracted African Star apple Juice; AFE -African Star apple juice stored at ambient temperature; RDE - African Star apple juice stored at refrigeration temperature

Phytochemical Properties of African Star Apple Juice during Storage

For the fruit juice at refrigerated temperature, the phenol content increased as storage time increased (Table 3). This could be attributed to the relative resistance of total phenolics during refrigerated storage (Zulueta *et al.* 2013). However, at ambient temperature, variations occurred in the phenolic content as the storage period increased. The degradation of total phenolics at ambient temperatures has been reported by various authors (Zheng and Lu 2011). The degradation of the phenol content could result partly from increased oxidation of phenolic substrate to quinone occasioned by high average ambient temperature of storage. Decreased synthesis of phenolic compounds in fruits and juices as a result of storage temperature fluctuations have been reported (Galani *et al.*, 2017). Phenolic compounds act as antioxidants by forming stable radical intermediates, preventing further oxidative processes in food products. Thus, amount of these compounds detected by analysis may be determined by the bound-status of different tissue fractions of fruit.

Reports have shown that flavonoids possess antioxidant, anti-tumour, anti-inflammatory, anti-allergic and anti-viral activities (Donald, 2000). Table 3 shows that flavonoid was observed to remain constant after first week of storage and then gradually increased till the end of the storage period at ambient temperature. However, at the refrigerated temperature, the flavonoid content remained steady till the end of the third week and thereafter increased at the end of the storage period. These findings were in opposition to the observation of Chikwendu *et al.* (2016) where the flavonoids were observed to decrease. The decrease was attributed to the fermentation and production of other compounds related to flavonoids. Also, Adeboyejo *et al.* (2019) attributed the degradation of flavonoid to activity of enzymes polyphenol oxidase and peroxidase, initiated and sustained by temperature, light, pH and reaction of other components in fruits and its product matrix.

Tannin content of the juice was also observed to increase as the storage period increased at both ambient and refrigerated temperatures. This increase could be associated with the

microbial fermentation that resulted into the production of increased levels of tannic acid in the juice.

Tannins are heat-stable, non-nutritive secondary metabolites and polyphenolic compounds known to have bitter, astringent tastes. In this study, there was a gradual increase in the tannin content as the storage period increased at both storage temperatures. This corroborates with the findings of Adeboyejo *et al.* (2019). The increase in tannin content on storage could be as a result of increased bio-accessibility of tannins in the chloroplast structure released through mechanical homogenization and pasteurization during processing. Also, irreversible oxidative transformation of pro-anthocyanidins, some flavonoids monomers and polyphenols to form new tannin-like compounds in the presence of polyphenol oxidase as catalyst may explain the increase in tannin content on storage.

Al Hassan *et al.* (2016) in their study of phytate in diets of pregnant women concluded that phytate is the strongest inhibitory predictor of mineral bioavailability as it is significantly associated with bioavailability of calcium, iron and zinc from diet. The phytate content of the ASA juice at ambient temperature was observed to increase till the end of 2nd week but later decreased till the end of the 4th week. On the other hand, the phytate content of the ASA juice at refrigerated temperature increased till the end of the first week but later decreased till the end of the 4th week. The decrease of phytate levels from the end of 2nd and 1st week for ambient and refrigerated storage, respectively is probably related to the capacity of endogenous phytates to be metabolized on incubation.

Although saponins are regarded as anti-nutrients in food, research evidences show that they have beneficial hypocholesterolemic effects in human diets because they form insoluble complexes with cholesterol, thereby inhibiting their absorption (Enujiugha *et al.*, 2014). The saponin content was observed to decrease with increase in the storage period at both storage temperatures. Saponins have been revealed to possess cholesterol lowering properties, which makes it beneficial to humans (Babarinde *et al.*, 2019).

Table 3: Phytochemical Properties of African Star Apple Juice during Storage

Storage Time (Weeks)	Sample	Phenol (mg/ml)	Flavonoid (mg/ml)	Tannin (mg/ml)	Phytate (mg/ml)	Saponin (mg/ml)
0	ABC	41.20±0.04 ^b	1.15±0.06 ^a	1.50±0.01 ^a	140.18±0.06 ^f	47.53±0.02 ⁱ
1	AFE	41.52±0.02 ^c	1.15±0.04 ^a	1.51±0.02 ^a	147.73±0.02 ^g	41.59±0.11 ^g
	RDE	41.44±0.06 ^c	1.15±0.06 ^a	1.56±0.01 ^b	157.97±0.05 ⁱ	41.79±0.05 ^h
2	AFE	40.95±0.08 ^a	1.22±0.02 ^{ab}	1.64±0.01 ^c	152.64±0.04 ^h	37.46±0.01 ^f
	RDE	41.43±0.01 ^c	1.13±0.01 ^a	1.82±0.04 ^d	132.55±0.01 ^e	36.75±0.04 ^e
3	AFE	42.62±0.50 ^e	1.45±0.05 ^c	1.98±0.01 ^e	110.51±0.02 ^a	30.55±0.05 ^d
	RDE	42.21±0.02 ^d	1.15±0.04 ^a	1.97±0.04 ^e	115.97±0.04 ^c	29.38±0.08 ^c
4	AFE	42.58±0.01 ^e	1.51±0.01 ^c	2.17±0.01 ^f	109.57±0.03 ^b	20.62±0.02 ^a
	RDE	42.76±0.04 ^f	1.28±0.01 ^b	2.19±0.01 ^f	120.24±0.04 ^d	21.76±0.04 ^b

Mean (±SEM) with different alphabetical subscripts in the same column are significantly different at p<0.05.

ABC- Refreshly extracted African Star apple Juice; AFE -African Star apple juice stored at ambient temperature; RDE - African Star apple juice stored at refrigeration temperature.

DPPH and Fe²⁺ Scavenging Abilities (%) of African Star Apple during Storage

The scavenging activity of DPPH was observed to undergo variations during the duration of storage at both storage temperatures (Fig. 1). ASA juice stored under ambient temperature was observed to display higher free radical scavenging capacity at the end of the storage period compared to the sample stored under refrigerated temperature. This increase might be associated with the increase in phenolic compounds which are natural antioxidants and have the ability to scavenge the free radicals, which cause damage to human health occasioned by oxidative stress (Martínez-Flores *et al.*, 2015). Dauda *et al.* (2022) also observed generally high antioxidant properties of some indigenous beverages (zobo

drink, kunun zaki, kunun aya and tamarind juice) commonly consumed in an urban neighbourhood in northern Nigeria.

Majorly, iron exists as haemoglobin of the red blood cells. It is known to play a major role in many parts of the body which includes, work performance, regulation of the body temperature, cognitive development and proper function of the immune system (Chikwendu *et al.*, 2016). In this study, it was observed that the iron content at both ambient and refrigerated storage decreased progressively. This is in agreement with the findings of Chikwendu *et al.* (2016) for unripe pawpaw juice. This decrease could be as a result of the chelation of iron by phytate and tannins in the fruit juice.

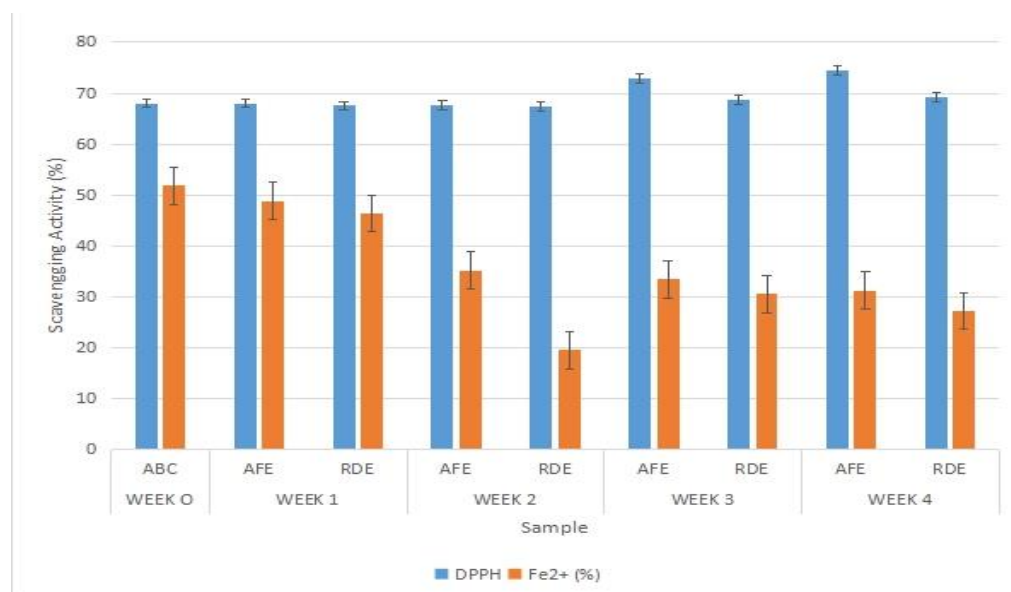


Figure 1: DPPH and Fe²⁺ Scavenging Abilities (%) of African Star Apple during Storage

ABC- Freshly extracted African Star apple Juice; AFE -African Star apple juice stored at ambient temperature; RDE - African Star apple juice stored at refrigeration temperature

Changes in FRAP and Vitamin C during Storage of African Star Apple Juice

FRAP assay quantifies the total reducing capability of antioxidants as a measure of the total antioxidant power in which the antioxidants act as reductants in a redox colorimetric reaction, releasing hydrogen atom to the ferric complex produced to discontinue the radical chain reaction (Giwa and Enujiugha, 2021). The antioxidant capacity of fruits and vegetables, which benefits human health, is highly correlated with their anthocyanin and total phenolic content (Enujiugha *et al.*, 2014). The present results indicating increasing FRAP levels with storage duration (Fig. 2) is contradictory to the findings of Mgaya-Kilima *et al.* (2014) in roselle-fruit juice blends where FRAP values were not increasing during storage. The decrease was attributed to the possibility of formation of polymeric compounds from monomeric anthocyanins during storage which were able to compensate the loss of antioxidant capacity due to decreased monomeric anthocyanins (Brownmiller *et al.* 2008).

Vitamin C is known to play a significant role in the metabolism of the body, healing of wounds, haemoglobin synthesis and intracellular cement substance (Badejo *et al.*, 2016). Foods containing ascorbate are known to undergo oxidation

(Chikwendu *et al.*, 2016). In this study it was observed that the increase in storage periods led to the decrease in the vitamin C content of the fruit juice. This is similar to the observation of Chikwendu *et al.*, (2016) on storage effect of unripe pawpaw juice. The retention of vitamin C is usually used to estimate the overall nutrient retention present in a food product because vitamin C is one of the least stable nutrients in foods. According to Davey *et al.* (2000), it is sensitive to oxidation and leaches into water soluble media during storage. Reports have shown that ascorbic acid is very sensitive to oxidation and converted to dehydroascorbic acid by the enzyme ascorbinase (Bhardwaj and Nandal, 2014).

The decrease in pH was lower under refrigerated storage condition which may be attributed to low temperature and high relative humidity in storage, which inhibited the conversion of acid in sugars and decreased rate of ascorbic acid oxidation which is highly dependent on the pressure of oxygen in the head space or dissolved in the juice (Costa *et al.*, 2003). These results are supported by the observations of Bhardwaj and Nandal (2014) in Kinnow Mandarin juice blends and Dauda *et al.* (2017) in African star apple juice. Immediately after harvest, ascorbate starts to degrade and steadily continues as the storage period prolongs (Murcia *et al.*, 2000) and even in

frozen foods (Rickman *et al.*, 2007). The presence of vitamin C in juice helps to improve the absorption of iron into the body system. It reduces and chelates nutrients during food digestion. On the other hand, the role of ascorbic acid in the prevention

of diseases related to oxidative damage is largely due to its ability to neutralize the action of free radicals in biological systems (Badejo *et al.*, 2016).



Figure 2: Changes in Vitamin C content and FRAP during Storage of African Star Apple Juice
 ABC- Refreshly extracted African Star apple Juice; AFE -African Star apple juice stored at ambient temperature; RDE - African Star apple juice stored at refrigeration temperature

Microbial Counts in African Star Apple Juice

The increase in the microflora of the African star apple juice (Table 4) was directly attributed to the storage period and temperature, as well as the high moisture that enabled suitable breeding ground for the growth of microorganisms and subsequent spoilage. The growth rates of microorganisms were lower in juice samples stored at refrigerated temperatures compared to those stored at ambient temperatures. This may be attributed to the ability of low temperatures to inhibit the growth of microorganisms in foods (Krishnakumar *et al.*, 2013) while high temperatures favour the growth of microorganisms. Yeast and mould growths are favoured by the presence of sugar and acid pH, which consequently predispose infected foods to attack by bacterial pathogens (Abbo *et al.*,

2006; Ezeama, 2007; Okwulehie and Alfred, 2010). Generally there were less total bacterial, mould and yeast growths in ASA juice stored at refrigerated temperature than at ambient conditions.

The absence of faecal coliforms in both juices could be attributed to good hygiene condition of juice during processing and storage. The absence of the main faecal coliform, *Escherichia coli*, is indicative of the absence of faecal contamination, which could be traced to the state of the portable water used during preparation in a bid to improve the safety of fresh fruit and vegetables. This is a pointer to hygienic production techniques and handling practices employed (Enujiugha, 2020).

Table 4: Microbial Count of Stored African Apple Juice during Storage (cfu/ml)

Weeks	Samples	Total viable bacteria count	Total coliform count	Total viable yeast and mould count	Total fecal coliform count
0	ABC	2.7 x 10 ⁴	1.1 x 10 ⁴	3 x 10 ³	Nil
1	AFE	5.2 x 10 ⁴	1.7 x 10 ⁴	9 x 10 ³	Nil
	RDE	3.3 x 10 ⁴	1.5 x 10 ⁴	5 x 10 ³	Nil
2	AFE	9.1 x 10 ⁴	2.7 x 10 ⁴	1.5 x 10 ⁴	Nil
	RDE	5.6 x 10 ⁴	1.6 x 10 ⁴	7 x 10 ³	Nil
3	AFE	1.37 x 10 ⁵	4.6 x 10 ⁴	2.2 x 10 ⁴	Nil
	RDE	6.2 x 10 ⁴	2.1 x 10 ⁴	9 x 10 ³	Nil
4	AFE	6.6 x 10 ⁴	3.4 x 10 ⁴	9 x 10 ³	Nil
	RDE	1.84 x 10 ⁵	8.2 x 10 ⁴	1.1 x 10 ⁴	Nil

Mean (±SEM) with different alphabetical subscripts in the same column are significantly different at p<0.05.

ABC- Freshly extracted African Star apple Juice; AFE -African Star apple juice stored at ambient temperature; RDE - African Star apple juice stored at refrigeration temperature

Sensory Evaluation of Fresh and Stored African Star Apple Juice

Panels' responses presented in Table 5 revealed that the juice significantly differed in appearance, taste, clarity and overall acceptability of the juice over storage time and conditions. However, the decreasing rates of these attributes were lower for stored juice at refrigerated temperature compared to the samples stored at ambient temperature. This might be as a result of the occurrence of fermentation during the storage periods and conditions to result into the production of undesirable microflora spoilage which caused the deterioration of the sensory attributes (Chikwendu *et al.*, 2016). Also, a possible explanation for better sensory evaluation of juice blends when kept in refrigerated storage might be as a result of the low temperature and high relative humidity did not cause any change in qualitative characters and palatability of stored juice and helped in maintaining juice appearance, flavour and total soluble solids than the ambient storage condition. In

ambient condition change in appearance of ASA juice might be attributed to oxidation of phenolic compounds present in juice and chemical reactions apparently follow the formation of dark pigments. Similar findings were reported earlier by Bhardwaj and Nandal (2014) and Prasad and Mali (2000) reported on Kinnow mandarin juice blends and pomegranate squash, respectively remained better at low temperature than at room temperature.

Also, the decrease in the clarity of the juice over storage period at both storage temperatures may be as a result of the increase in the total soluble solids. According to Mirhosseini *et al.* (2008), it was discovered in the study carried out on influence of CMC and pectin on physical stability, turbidity loss rate, cloudiness and flavour release of orange beverage emulsion during storage. The ASA juice stored at ambient temperature was more cloudy compared to the one stored at refrigerated temperature.

Table 5: Sensory Evaluation of fresh and Stored African Star Apple Juice

	Sample	Appearance	Flavour	Clarity	Taste	Overall Acceptability
Week 0	ABC	9.26±0.05 ^a	7.65±0.81 ^a	8.34±0.51 ^a	8.37±1.07 ^a	8.85±1.03 ^a
	AFE	7.52±0.2 ^{bc}	4.26±1.01 ^b	6.26±0.68 ^b	5.28±1.03 ^c	7.22±1.11 ^{bc}
Week 1	RDE	8.75±0.12 ^b	6.48±0.74 ^a	7.82±0.41 ^a	7.31±1.09 ^b	7.53±1.08 ^b

Mean (±SEM) with different alphabetical subscripts in the same column are significantly different at $p < 0.05$.

ABC- Refreshly extracted African Star apple Juice; AFE -African Star apple juice stored at ambient temperature; RDE - African Star apple juice stored at refrigeration temperature

4. Conclusion

The results obtained in this research revealed that extracted African star apple (ASA) juice stored under refrigeration temperature had a shelf life of up to 2 weeks; this can help in the reduction of post-harvest losses of this tropical fruit and increases the market value. Ambient conditions of storage would not preserve the freshness of the juice beyond one week. There is a high potential for African star apple fruit to serve as a disease preventing source for mankind due to the high availability of phytochemicals, antioxidants and nutrients in its cotyledons.

Ethical Approval

This study was considered and approved by the Ethics Committee of the School of Agriculture and Agricultural Technology, Federal University of Technology, Akure, with assigned number FUTA/SAAT/ETH/2020/007.

CRedit authorship contribution statement

VNE & AOA: Conceptualization, Initial draft, Editing, Final review. TTA & OAA: Laboratory analysis, Initial draft. SAF, CBG & MOO: Editing, Referencing, Final review.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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