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ANTIOXIDANT AND PHYSICOCHEMICAL PROPERTIES OF *Chrysophyllum albidum* FRUIT AT DIFFERENT RIPENING STAGES

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ABSTRACT

Chrysophyllum albidum (African Star Apple) fruit is an indigenous African fruit. The African Star Apple plant popularly referred to as the "alasa" tree grows widely in tropical Africa. Ripening influences biochemical processes and subsequently affects the nutritional and bioactive characteristics of the fruits. This study sampled *Chrysophyllum albidum* fruit of three maturity stages and determined the colour, proximate, total phenol and antioxidant characteristics of the fruits. Spectrophotometric methods were used in the determination of total antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, 2,2-azinobis,3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and Ferric reducing ability of plasma (FRAP) assay. Significant differences ($p<0.05$) were observed in the colour of the fruit across all the ripening stages. The ripe stage of the fruit recorded the highest extent of lightness among all the other ripening stages. Both the ripe and over-ripe fruits appeared reddish in colour with the ripe fruit demonstrating a higher red intensity than the over-ripe fruit. The calculated metric chroma of the *Chrysophyllum albidum* fruits were in the range of 2.33-21.67 for the three ripening stages. Hue angle values recorded were in the range 46.46-92.00. Browning and colour indices for fruits of all the stages of development were of range 6.40-80.30 and -14.30-16.96, respectively. Proximate analysis of the three fruit categories showed that the unripe fruits had higher carbohydrate (69.27 %), crude protein (8.17 %), and crude fat (7.99 %) content relative to fruits which were ripe and over-ripe. The over-ripe fruit had higher crude fibre (7.36 %) and ash (3.86 %) content. Total phenolic content for the unripe fruit (20µg GAE/mg) was also higher than fruits of the other ripening stages. The antioxidant ability of the *Chrysophyllum albidum* fruit showed that the unripe stage recorded the highest DPPH (29.24 %) and ABTS (99.09 %) radical scavenging activity. The ferric reducing antioxidant potential of the fruit at different ripening stages was significantly different ($p<0.05$) with the unripe stage recording the highest potential. The unripe fruits demonstrated higher proximate and antioxidant composition than the ripe and over-ripe fruits. Extracts from the unripe fruit can serve as useful nutraceuticals in functional food formulations.

Key words: *Chrysophyllum albidum***,** bioactive compounds, antioxidant activities, nutritional properties, colour characteristics

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INTRODUCTION

Fruits are rich sources of essential nutrients, dietary fibre, antioxidants and bioactive compounds that appreciably lower the risk of colorectal cancer, diabetes, hypertension, coronary heart diseases and stroke [1, 2]. Wild fruits are important in the diets of the rural poor as they provide a cheap source of energy, proteins, fats, vitamins and minerals which contribute significantly to food and nutrition security [3].

Chrysophyllum albidum commonly referred to as "alasa" tree in local Ghanaian parlance normally grows wild. This plant is found in the tropical regions of Africa and belongs to the family Sapotaceae [4]. The fruit of *Chrysophyllum albidum* is seasonal and contains about 4 to 5 brown seeds [5, 6]. Fruiting begins in July and ripen between December and March. The fleshy pulp of the *Chrysophyllum albidum* fruit which has sweet-sour taste is widely eaten by indigenous people and used for fruit jams [7, 8]. The juicy fruit is used for the production of soft drinks and the seed is a potential source of oil [9]. *Chrysophyllum albidum* has also been used by local communities for medicinal purposes [7]. The bark of the *Chrysophyllum albidum* tree, its leaves, roots, fruits and seeds have been reported to be of medicinal value and used in the treatment of conditions like malaria, sterility and hemorrhoids [7]. The fruit is also known to contain high amounts of anarcardic acid which is very useful in industry for the protection of wood [9]. Gum extracts from the fruit have good physicochemical properties and have been used in the development of drug formulations as pharmaceutical excipients [10].

Fruit ripening is characterized by varied biochemical processes such as changes in fruit colouration, soluble sugar, total acidity and sensory characteristics that influence fruit consumption [11]. The ripening process has long been known to impact nutritional and bioactive characteristics of various produce such as tomato, banana and plantain, cherry fruits, sapota-do-Solimoes fruit, Myrtaceae fruits and Feijoa fruits [1, 11, 12, 13, 14]. However, information on the nutritional and bioactive components of the *Chrysophyllum albidum* fruit at different ripening stages is scarce. This study therefore, was conducted to determine the effect of maturity stage of the fruit on proximate and bioactive properties to provide useful information on the nutritional and antioxidant potential of the fruit.

MATERIAL AND METHODS

Materials

Sodium nitrate, Aluminum chloride, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and Gallic acid were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Folin-Ciocalteu reagent was obtained from Merck (Darmstadt, Germany) with sodium bicarbonate from May & Baker Chemicals (Dagenham, E.). All the chemicals that were used were of analytical grade.

Source of *Chrysophyllum albidum* Fruit

Fresh *Chrysophyllum albidum* fruits of three maturity stages were obtained from a farm at Bame in the Ho West district of the Volta Region of Ghana. The region spans

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between latitudes 6.33 ° 32" N and 6.93 ° 63" N and longitudes 0.17° 45" E and 0.53 ° 39" E [15]. The study area has a bi-modal climatic condition with two rainy seasons referred to as the major and minor seasons. The major season is from March to June while the minor one is from July to November. Mean annual rainfall figures are between 120.1 mm and 192 mm. The highest rainfall occurs in June and has mean value of 192 mm while the lowest rainfall is in November recording about 120.1 mm [15]. Annual mean temperature and humidity range from 22 to 32 °C and 68 % to 83 % respectively, whereas annual mean sunshine hours range from 319 to 371 h.
Three independent samplings of fruit were carried out and represented three different ripening stages (Figure 1). The different sampling stages were studied to observe how the maturity stage of fruit influenced colour, proximate, total phenol and antioxidant characteristics. The unripe fruits were harvested in September, 2018 (first stage) and ripe fruits were harvested in December, 2018 (second stage). The third stage of ripening was achieved by keeping ripe fruits at room temperature (25 °C) in the laboratory for 7 days to over ripe.

Figure 1: *Chrysophyllum albidum* fruits sampled in the study (a) Unripe fruits (b) Ripe fruits (c) Over-ripe fruits

Colour Determination

Measurement of colour indicators (L* a* b*) of the *Chrysophyllum albidum* fruits were done using the Chroma meter (CR-310 Minolta Camera Co., Osaka, Japan). L* is a colour parameter that measures the extent of lightness, a* indicates redness of sample, and b* value when positive signifies a yellowish colour coordinate. The colorimeter was standardized using a white tile and colour measurements were taken in triplicates on the surface of the *Chrysophyllum albidum* fruits.

Calculated values for the hue angle (Ho) and metric Chroma (C) were determined using the colour parameters- a* and b* as follows [16]:

$$\text{Hue angle } (^{o}) = \tan^{-1}(b^*/a^*) \quad (1)$$

When a* < 0, Hue angle = $180 + \tan^{-1}(b^*/a^*)$

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

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The change in colour relative to the control (ΔE) was determined as follows;

$$\Delta E = [(Lo-L^*)^2 + (ao-a^*)^2 + (bo-b^*)^2]^{1/2} \quad (3)$$

where:

Lo, ao and bo are initial values of fruits.

L*, a*, and b* are final values.

$$\Delta a^* = a^*o \text{ control} - a^* \text{ sample} \quad (4)$$

$$\Delta b^* = b^*o \text{ control} - b^* \text{ sample} \quad (5)$$

$$\Delta L = L^* \text{ control} - L^* \text{ sample} \quad (6)$$

$$\Delta H = Ho \text{ control} - Ho \text{ sample} \quad (7)$$

$$\Delta \text{ Chroma} = \text{Chroma control} - \text{Chroma sample} \quad (8)$$

The Browning and Colour indices (BI and CI) were also calculated using values obtained for L*, a* and b* [17] as follows:

$$\text{Browning Index (B.I.)} = \frac{[100\,(x-0.31)]}{0.17} \quad (9)$$

where:

$$X = \frac{(a^* + 1.75\,L^*)}{(5.645\,L^* + a^* - 3.012b^*)}$$

$$\text{Colour Index (C.I.)} = 2000 \times a^*/L \times [a^{*2} + b^{*2}]^{1/2} \quad (10)$$

Preliminary Sample Preparation

Twenty (20) fruits per each ripening stage were washed and the seeds removed manually using a knife to obtain the pulp portions together with the skin. The edible pulp together with the skin of the fruit which has been shown to be rich in fibre, minerals and ascorbic acid was oven-dried at 55°C for 6 h (Binder Heating and Drying Oven, Tuttlingen, Germany) [18, 19]. The dried samples were size reduced (Thomas scientific mini-miller; Model 3383-L70) and a 400/425 µm pore-size laboratory sieve was used to obtain fine powder. The powdered samples were packaged in ziploc bags and stored in a freezer (Protech PRCF-500, China) at -20 °C.

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Proximate Composition of *Chrysophyllum albidum* Fruits

Proximate composition of the fruit was carried out using Association of Official Analytical Chemists methods, to determine the moisture, ash, crude fat, crude fibre, crude protein and carbohydrate contents of the fruits [20].

Antioxidant and Total Phenol Determination

Methanolic extracts of the milled samples were prepared by weighing 20 g of the milled sample for each ripening stage into a conical flask and 100 ml methanol (70 % v/v) was added [21]. The mixture was allowed to stand at room temperature for 48 h with periodic manual shaking, after which the mixture was filtered using 125 mm filter paper to remove solid particles. The filtrates were then transferred into pre-weighed petri dishes and then evaporated at 45°C in an oven (Binder Heating and Drying Oven, Tuttlingen, Germany) to remove the methanol. The extracts obtained after drying were then used for total phenol and antioxidant determinations.

Determination of Total Phenol Content

Total phenol content was calorimetrically determined using Folin Ciocalteu reagent [22]. About 5 mg of each extract was dissolved in 1000 µl of methanol and vortexed to obtain a concentration of 5 mg/ml. Thereafter, 200 µl of the 5 mg/ml solution was dissolved with 800 µl of distilled water to obtain 1 ml of a solution in a test tube. Distilled water (5 ml) and Folin Ciocalteu's reagent (0.5 ml) were added to the resulting mixture and vortexed. Sodium carbonate (20 %; 1.5 ml) was added to the mixture and the volume made up to 10 ml with distilled water. The mixture was incubated for 2 h (25°C) after which absorbance was measured at 750 nm (Jenway Vis Spectrophotometer, 6305; Cole-Parmar, Staffordshire, UK). The total phenol content of each sample was determined in Gallic Acid Equivalent (GAE) mgg^{-1} on sample dry weight basis.

Determination of Antioxidant Activity using DPPH (1, 1-diphenyl–2 picrylhydrazyl) Radical Scavenging Activity Assay

The 1, 1-diphenyl–2 picrylhydrazyl **(**DPPH) scavenging activities of the extracts obtained were determined as described previously [23]. The stock DPPH (150 µl) was diluted with 99 ml of distilled water. A sample volume of 200 µl was pipetted into a test tube and 800 µl of DPPH reagent was added and shaken for 5 min. The mixture was left at room temperature for 30 min and the absorbance was read at 517 nm using the spectrophotometer (Jenway Vis Spectrophotometer, Cole-Parmar, Staffordshire, UK 6305). The radical scavenging capacity was calculated as percentage of the control sample (free radical solution minus extract) as:

$$(\%)\ \text{inhibition} = \{[(Ac - At)/ Ac] \times 100\} \qquad (11)$$

where: Ac is the absorbance of control; At is the absorbance of the extract

Determination of Antioxidant Activity using ABTS (2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic)

The 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic) (ABTS) acid scavenging activity was determined as per protocol reported previously [24]. The 2, 2'-azino-bis

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(3-ethylbenzthiazoline-6-sulphonic) (ABTS) (7 mM, 10 ml) and potassium persulfate (2.4 mM, 10 ml) stock solutions were prepared and mixed. The mixture was incubated in the dark (25°C; 12 h) and an aliquot (1 ml) subsequently diluted with 50 ml of methanol. The dissolved extract (200 µl) was mixed with the ABTS free radical solution (800 µl), incubated (30 °C; 10 min and absorbance measured at 734 nm (Jenway Vis Spectrophotometer, 6305; Cole-Parmar, Staffordshire, UK). ABTS• + scavenging activity was determined relative to BHA and inhibition was calculated as follows:

$$(\%)\ \text{inhibition} = \{[(Ac - At)/ Ac] \times 100\} \quad (12)$$

where: Ac is the absorbance of control; At is the absorbance of the extract

Determination of Antioxidant activity using FRAP (Ferric Reducing Antioxidant Potential) Assay

Ferric reducing power of the extracts of *Chrysophyllum albidum* fruits was determined with FRAP assay [25]. Ferric Reducing Antioxidant Potential (FRAP) reagent was prepared using acetate buffer (10 ml of 300 mM, pH 3.6), 1 ml of 10 mM TPTZ (2,4,6-tri(2-pyridyl)-s-triazine) in 40 mM HCl and 1 ml of 20 mM ferric chloride. The solutions were freshly prepared before use. Thereafter, 100 µl of each extracted sample was added to 3 ml of freshly prepared FRAP reagent. The reaction mixture was placed in a water bath (37 °C; 30 min) and absorbance was measured at 593 nm (Jenway Vis Spectrophotometer, 6305; Cole-Parmar, Staffordshire, UK). Ferric Reducing Antioxidant Potential (FRAP) value was determined as the difference between the absorbances of sample and the blank.

Statistical analysis

Statgraphics (Graphics Software System, STCC, Inc. USA) was used to analyse data. Comparisons between the different samples were done using analysis of variance (ANOVA) and differences between means were determined with LSD at 5% level of significance ($p<0.05$). All measurements were calculated from the value obtained from duplicate assays.

RESULTS AND DISCUSSION

Colour of *Chrysophyllum albidum* Fruit at Different Ripening Stages

Result for the colour determination of *Chrysophyllum albidum* fruit at different ripening stages are shown in **Table 1**. Although there was no significant difference ($p>0.05$) between the unripe stage and the ripe stage of the fruit in terms of lightness, results indicated a significant difference ($p<0.05$) in lightness between the unripe stage and the over-ripe stage. The ripe stage recorded the highest extent of lightness among all the other ripening stages. The colour of agri-food produce changes as the fruit or vegetable ages [26]. The primary pigments imparting colour quality are the fat-soluble chlorophylls (green), carotenoids (yellow, orange, and red), water-soluble anthocyanins (red, blue), flavonoids (yellow) and betalains (red) [26]. The parameter “a” could either be positive, indicative of redness or negative, indicative of greenness. The results for the “a” parameter revealed significant differences in the colour of the fruit across all the

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ripening stages with the unripe stage recording a negative "a" value and both the ripe and over- ripe stages recording a positive "a" value. The fruit in the ripe stage had a higher "a" value than the over-ripe stage. The negative "-a" value recorded by the unripe fruit confirms its greenish colour. Both the ripe and over-ripe stages of ripening appeared reddish in colour with the ripe fruit demonstrating a higher red intensity than the over-ripe stage. The "b" value for all the ripening stages was positive, indicating that the fruit at all ripening stages was more yellowish. Fruits undergo significant textural and colour transformation as they pass through the different stages of ripening. The colour conversion from the unripe to ripe stage usually spans from dark green to pale green and then to slight yellow or orange colour [26]. This colour transformation is attributed to chlorophyll degradation which subsequently reveals the yellow carotenoid pigments. There was an increase in L* values from unripe to ripe state of maturation but did not differ significantly ($p>0.05$) which suggests a brightening of the initial colour.

The calculated metric chroma of the *Chrysophyllum albidum* fruits were in the range of 2.33-21.67 for the three ripening stages. There was an observed upsurge of saturation from the unripe to ripened stage which then decreased during the overripe stage. The colour saturation of the initial stage (unripe) differed ($p<0.05$) from the other two stages. Hue angle values recorded were in range 46.46-92.00. There was a reduction in angles from the initial (unripe) to about half of its value during the process of transition from the unripe to ripe stage. A lower hue value implied a redder product while a greater value implied a bluer or purple product. Browning and colour indices for *Chrysophyllum albidum* fruits of all the stages of development were in range 6.4-80.3 and -14.3-16.96 respectively. Statistically, there were significant differences ($p<0.05$) recorded. Browning may have occurred due to Maillard reactions during storage which implied increased enzymatic reaction. An association between L* and a* to the degree of browning had been observed by Sapers and Douglas [27]. Browning of the pulp of the fruits was possibly linked to the concentrations of phenolics in ripe fruit as suggested by Diaz-Perez *et al*. [28].

The total colour difference (ΔE) designates the scale of the difference between locations in the CIE L* a* b* colour system. Hence, the overall change in colour for unripe to ripe and ripe to over ripe were 20.28 and 15.01 respectively (Table 2) which represents a greater change in colour relative to the initial. An upsurge in the carotenoid content of a fruit is one of the most noticeable characteristics of ripening of the fruit [29]. The color change in fruits from green to orange during ripening is due to chlorophyll degradation and carotenoid accumulation [26]. Reddish orange color is caused by high concentration of lycopenes and carotenoids. After reddish/orange ripe, the last stage is the process of senescence which involves degradation of cellular tissue eventually leading to the death of the cells. Furthermore, there is an increase in respiration, increase in ethylene production, fruit acidity changes, and changes in starch and sugar content are some major biochemical changes that occur during ripening which influences the development of color following a typical sequence with ripening. In a related study, H=52, C= 45 and L* =60 were respectively reported for hue angle, metric chroma and whiteness during ripening and storage of sapote mamey (*Pouteria sapote* (Jacq.) HE and Stern) fruits [28].

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Proximate Composition of *Chrysophyllum albidum* Fruit at Different Ripening Stages

The proximate composition of *Chrysophyllum albidum* fruit (pulp together with the skin) at the different ripening stages presented on dry weight basis showed significant differences (p<0.05) (Table 3). The moisture content of the ripe *C. albidum* fruits (11.26 %) was higher than the unripe (4.18 %) and over-ripe (9.48 %) fruits. This could imply that the moisture content of the fruit increases with ripening however, declined after ripening (over-ripe stage). The implication is that unripe fruits can be stored for a longer time than the ripe and over-ripe fruits [6]. Other studies have also shown an increase in moisture content with ripening and this is attributable to the breakdown of cells as maturity progressed [30]. The moisture contents of the ripe and overripe fruits were higher than values obtained for *C. albidum* fruit pulp (7.76 %) and skin (4.80 %), and African star apple seeds (8.49 %) on dry weight basis [19, 31]. The carbohydrate content of African star apple ranged from 65.55 % (ripe fruit) to 69.27 % (unripe fruit). These values were lower than the carbohydrate content of African star apple supplement (70.90 %) and dried mango slices (73.91 %; full ripe – 78.95; unripe) [4, 32]. The carbohydrate content of unripe fruits of *Chrysophyllum albidum* was higher than the ripe and over-ripe fruits, this implies that as the fruit ripens the carbohydrate content of the fruit decreases [33]. Unripe fruits usually contain carbohydrates in the form of starch which gets converted to sugar as the fruit ripens [6]. The sugar is further converted to ethanol at the over-ripe stage. However, contrary to what was observed in *C. albidum* fruits, total carbohydrates in noni (*Morinda citrifolia*) fruits increased with ripening [30]. Hence changes in the nutritional components of fruits at different ripening stages could be species-specific. Analysis of the ash content of the fruit showed an increasing trend with ripening and values obtained (3.67 %; unripe fruit to 3.86 %; overripe fruit) were higher than the reported values for unripe fruit (0.63 %) and ripe fruits (0.72 %) [6]. Increased levels of ash with ripening have been reported for *Chrysophyllum albidum* fruit [6] and mango fruit [32]. The protein content of the *Chrysophyllum albidum* fruit decreased with ripening and a similar pattern has been observed previously in the same fruit (*C. albidum)* obtained from Nigeria [6]. Protein values (6.08 – 8.17 %) obtained in this study were higher than values reported for dried mango fruits (4.01 – 4.90 %). The fat content of the fruit was high at the unripe stage, decreased with ripening as previously reported by Olufunke and Adeola [6]. However, the range of values obtained in this study (5.55 % to 7.99 %) were comparatively higher than the reported 0.19 % to 1.21 % [6]. The crude fibre of the *C. albidum* fruits increased with maturity from 6.72% (unripe) to 7.36 % (over-ripe), comparatively higher than the reported values for unripe (2.20 %) and fully ripe (1.80 %) cherry fruits [12]. Crude fibre content of the cherry fruits, however, decreased with ripening [12]. The relatively high crude protein, fat and carbohydrate content of the unripe *Chrysophyllum albidum* fruit could potentially serve as a useful nutritive source for food and pharmaceutical formulations.

Total Phenolic Content of *Chrysophyllum albidum* Fruit

The total phenolic content of *Chrysophyllum albidum* fruit at different ripening stages expressed as Gallic acid equivalent per gram of sample (µg GAE/mg) on dry weight basis is shown in Figure 2. The total phenolic content of the unripe fruit (20.46 µg/mg) was the highest as compared to the ripe and over-ripe stages (10.14 to 11.57 µg/mg),

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indicating the potential of the unripe fruit as a good source of dietary antioxidants for optimal health. The degree of ripeness markedly influences the quantity of fruit phenolic compounds. The degradation of some phenolic compounds may be faster or slower than the biosynthesis of other phenolic compounds. Decreased total phenolic content from the unripe to ripe stages of a fruit had also been reported for *Nypa fruticans* and *Gala* apple [34, 35]. However, matured red colour tomato fruits showed higher polyphenol content than fruits in the green state [36]. There was no significant difference (p>0.05) in the total phenolic content of the ripe and over-ripe fruit.

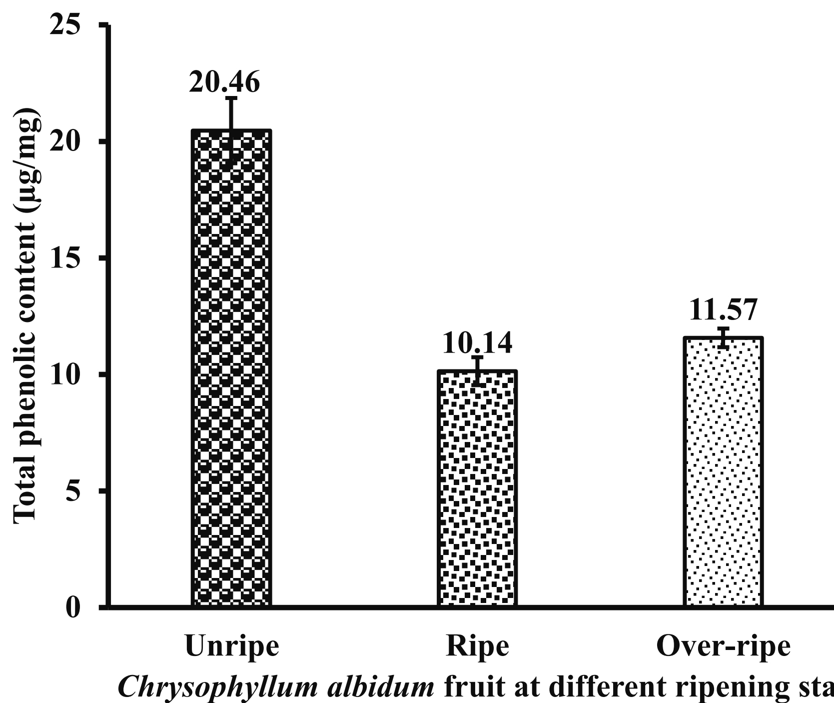

Figure 2: Total phenolic content of *Chrysophyllum albidum* fruit at different ripening stages

Antioxidant Activity of *Chrysophyllum albidum* Fruit

DPPH Scavenging Activity

The scavenging activity of the *Chrysophyllum albidum* fruit on DPPH is presented in Table 4. There was no significant difference (p>0.05) between the values obtained for the different ripening stages. The DPPH assay is commonly used for testing the radical scavenging activity of different fruits [37, 38]. Tropical fruits are rich in antioxidants which prevent free radical formation and various diseases associated with oxidative stress such as cancers and cardiovascular diseases.

In this study the observed activity against DPPH radicals increased from 17.71 % (ripe stage) to 29.24 % (unripe stage), suggesting a decrease in the natural antioxidant capacity as fruit ripening progressed. However, contrary to results obtained for the "alasa" fruit, DPPH radical scavenging activity increased as the maturity of cherry fruits advanced from un-ripened stage to fully-ripened stage [12]. The DPPH activity was found to be lower compared to reported values for *Chrysophyllum albidum* fruit

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pulp (92.4 %) obtained from Nigeria and a food supplement (86.23 %) developed from the African star apple fruit [4].

ABTS Acid Scavenging Activity

The results of antioxidant activity of the fruit based on ABTS assay are shown in Table 3. The results revealed that there was a significant difference ($p<0.05$) between the unripe stage and the other stages of ripening. However, there was no significant difference ($p>0.05$) between the ripe and over-ripe stage. The unripe stage thus, recorded the highest antioxidant activity (99.09 %), followed by the over-ripe stage (97.72 %) and finally the ripe stage (70.32 %). The high free radical scavenging capacity of the unripe *Chrysophyllum albidum* fruit demonstrates its potential health benefits of using unripe fruit in food formulations to improve the optimum health of consumers. The ABTS assay has also been used to compare the antioxidant activity of mature and immature acerola cherry extracts and the results showed that ABTS radical scavenging activity decreased from immature to mature stage [39]. The apparent decrease in vitamin C content during fruit development had been associated with the reduction in antioxidant activities from unripe to ripe [39].

Ferric Reducing Ability Potential (FRAP)

The results of the FRAP assay of the different samples are presented in Table 4. The assay is based on the reducing power of antioxidants present in extracts, indicating the potential of an antioxidant to reduce the ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) with the latter forming a blue complex (Fe2+/TPTZ). From the results (Table 4), it can be observed that the ferric reducing antioxidant potential of the fruit at different ripening stages was significantly different ($p<0.05$) and follows the order of increasing reducing power which was over-ripe stage (0.25 mg mL^{-1}) < ripe stage (0.28 mg mL^{-1}) < unripe stage (0.51 mg mL^{-1}). These results confirm that the unripe stage of the *Chrysophyllum albidum* fruit has the highest antioxidant activity. The ferric reducing power of the unripe fruit was also higher than for extracts from the seed and leaf of *Chrysophyllum albidum* fruit which showed values ranging between 0.128 and 0.479 mg mL^{-1}, with a greater activity observed in the leaf than seed extract [40].

CONCLUSION

Chrysophyllum albidum fruit showed significant differences for most proximate and bioactive constituents for the different ripening stages (unripe, ripe and over ripe), except for carbohydrate and ash content. The unripe fruits of *Chrysophyllum albidum* had higher protein, carbohydrate and fat values and lower ash and moisture values than the ripe fruits and over-ripe fruits. Since the unripe fruit is not freshly consumed, extracts of the beneficial nutritive constituents can be obtained and used as functional ingredients in food and pharmaceutical formulations.

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Table 1: Colour of *Chrysophyllum albidum* fruit at different stages of ripening

Ripening Stages	L*	a*	b*	Metric Chroma	Hue Angle (H°)	Browning Index (B.I.)	Colour Index (C.I.)
Unripe	35.60 ± 2.32[a]	-0.11±0.070[a]	2.34±0.68[a]	2.33	92.00	6.40	-14.30
Ripe	38.14 ± 0.74[a]	14.93±1.30[b]	15.71±1.62174[b]	21.67	46.46	80.30	16.96
Over-ripe	31.16 ± 2.24[b]	9.92± 1.67[c]	12.59±3.050[b]	16.03	51.76	73.50	10.20

Values are means ± standard deviations of triplicate determinations. Values in the same column having the same superscript letters are not significantly different ($p>0.05$)

Table 2: Fruit colour change upon storage of *Chrysophyllum albidum* fruits

Ripening Stage	ΔL*	Δa*	Δb*	Δ Metric Chroma	Δ Hue Angle	Δ Total Colour (E)
Unripe	–	–	–	–	–	–
Ripe	-2.54	-15.04	-13.37	-19.34	45.54	20.28
Overripe	4.44	-10.03	-10.35	-13.7	40.24	15.01

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Table 3: Proximate composition of *Chrysophyllum albidum* fruit at different stages of ripening on dry weight basis

Proximate Composition	Ripening stages		
	Unripe [%]	Ripe [%]	Over-ripe [%]
Carbohydrate	69.27 ± 0.61^{b}	65.55 ± 0.29^{a}	67.67 ± 0.73^{b}
Crude protein	8.17 ± 0.31^{b}	6.46 ± 0.49^{a}	6.08 ± 0.45^{a}
Crude fat	7.99 ± 0.53^{b}	7.56 ± 0.66^{ab}	5.55 ± 0.73^{a}
Ash	3.67 ± 0.03^{a}	3.71 ± 0.01^{a}	3.86 ± 0.03^{b}
Moisture	4.18 ± 0.42^{a}	11.26 ± 0.77^{c}	9.48 ± 0.13^{b}
Crude fibre	6.72 ± 0.44^{ab}	5.46 ± 0.27^{a}	7.36 ± 0.61^{b}

Percentage proximate compositions (dry weight basis) presented are means of duplicate determinations ± standard deviation. Values in the same row having the same superscript letters are not significantly different ($p>0.05$)

Table 4: Antioxidant activity of *Chrysophyllum albidum* fruit at different stages of ripening

Ripening stage	ABTS activity [%]	DPPH activity [%]	FRAP [mg mL^{-1}]
Unripe	99.09 ± 0.79^{a}	29.24 ± 7.48^{a}	0.51 ± 0.00^{a}
Ripe	70.32 ± 4.40^{b}	17.71 ± 8.13^{a}	0.28 ± 0.00^{b}
Over-ripe	97.72 ± 2.09^{a}	27.10 ± 2.77^{a}	0.25 ± 0.00^{c}

Concentrations presented are means ± standard deviation of triplicate determinations with different alphabets in columns showing significant difference ($p<0.05$). ABTS-2,2-azinobis,3-ethylbenzothiazoline-6-sulfonic acid; DPPH-1,1-diphenyl-2-picrylhydrazyl; FRAP-Ferric reducing ability of plasma

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