Phytochemical components of beverages from African star apple (*Chrysophyllum albidum*) tissue fractions under ambient storage

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There is increasing awareness of functional potentials of African star apple (ASA) as an underutilized tropical fruit, yet little is known of alterations to its product properties during storage. Changes in antioxidant and antinutritional composition of beverages derived from ASA peel, pulp and seed coat fractions during 8-week storage at 26°C were investigated. Drinks derived from the peel were registered and retained significantly higher concentration of vitamin C (0.53 mg/ml, 45%) than pulp (0.17 mg/ml, 6%) and seed coat (0.35 mg/ml, 9%) drinks. Pulp fraction had higher initial flavonoid and total phenolic contents (6.44 mg/100 ml and 13.37 mg/ml) than peel (3.41 mg/100 ml and 3.05 mg/ml) and seed coat fractions (3.65 mg/100 ml and 3.04 mg/ml) but decreased significantly at 92 and 93%, 90 and 67% and 81 and 67% respectively. Beverages derived from the peel, pulp and seed coat showed 98.67, 75.30 and 76.97% DPPH and FRAP of 22.20, 10.90 and 11.87 μmol/ml respectively. A continuous decrease in oxalates and saponin, and slight increase in tannin and phytate levels were observed in all samples throughout the storage period. Findings confirmed the functional potential of ASA tissue beverages as rich sources of natural antioxidants. Effect of ambient storage on stability of studied bioactive compounds was found to be significant and may be a possible indication of product shelf life.

Key words: Bioactive components, antioxidant activity, anti-nutritional factors, *Chrysophyllum albidum*, storage stability.

INTRODUCTION

A rich diversity of edible fruit species make up part of the daily diet and provide nourishment to millions of people. Some indigenous tropical fruits especially those of Sapotaceae family (*Chrysophyllum albidum*, *Synsepalum dulcificum*, *Vitellaria paradoxa*) are rich sources of natural dietary bioactive compounds such as vitamin C, anthocyanin, flavonoids, phenolic acids etc. The protective effect of fruits in inhibiting or delaying oxidative stress in ageing and the reduced risk of chronic diseases have been attributed to the antioxidant potentials of their bioactive components (Arueya and Ugwu, 2017). However, they are largely underutilized due to little or
no value addition to the fresh fruits resulting in high post-harvest loss and accompanying environmental pollution at their annual peak season periods. Processing into drinks and juices for consumption are practical ways of extending shelf-life of fruits at household and commercial levels while conserving their inherent micronutrient components (Aguilar et al., 2017). The production processes however may involve heat treatments which accelerate undesirable degradation of inherent bioactive compounds (Andrés et al., 2016). Sensitive essential micronutrients in fruit juices may be degraded under process and storage conditions and consequently may be an indicator of their nutritional quality during storage.

African star apple (ASA) also known as ‘Agbalumo’ or ‘Udara’ (Nigeria), ‘Azongogwe’ (Benin) and ‘Alaso’ (Ghana) is a non-climacteric fruit widely distributed in the rain forest zones of Africa. The use of its parts in ethno-traditional medicine have been corroborated by research evidences and found to possess hepatoprotective, anti-plasmodial and pathological activities (Abiodun et al., 2011; Adewoye et al., 2010). Aqueous extract of ASA pulp inhibited resistant Staphylococcus aureus strains at higher concentrations than ciprofloxacin, a synthetic antibiotic (George et al., 2018). Nutritionally, ASA pulp possesses higher vitamin C content at 446 mg/100 g than mango, pineapple, pawpaw and hog plum at 98.0, 38.3, 39.3 and 10.1 mg/100 g respectively (Edem et al., 1984; Ellong et al., 2015). Proximate composition and antioxidant activity of ASA follow a tissue distribution with a higher radical scavenging potential than common tropical fruits like pawpaw, pineapple and hog plum (Abiodun and Oladapo, 2011; Bello and Henry, 2015). Variations have been documented with anti-nutrients in ASA tissue fractions (Ibrahim et al., 2017). Acceptable single strength juice has been produced from ASA (Dauda et al., 2017).

During juice processing, peels and seed pericarp of the fruit are discarded as waste products but have been shown to contain higher nutritive constituents than pulp fractions (Abiodun and Oladapo, 2011). To the best of our knowledge, there are no reported investigations into the systematic comparison of antioxidant and anti-nutritional activities of beverages from parts of ASA fruit during ambient storage. Accordingly, this study aimed to elucidate influence of storage on antioxidant and anti-nutritional properties of drinks made from peels, pulp and seed coat fractions of ASA.

MATERIALS AND METHODS

Sample preparation

Ripe, firm ASA fruits (150 pieces) were collected from Oje fruit market in Ibadan, Nigeria during 2017/2018 harvest season. Fruits were sorted, washed with 0.15 g/L chlorinated water, rinsed with tap water and blanched at 80°C for 3 min to aid separation of peel from the pulp. Seed coats were removed from each seed. Juice was produced from pulp according to the method of Dauda et al. (2017). Peel and seed coat fractions were pulverized separately (Marlex CM/L7962804 food liquidizer, KIL, DAMAN) with activated carbon filtered tap water (30% w/v). The mixtures were filtered using muslin cloth, pasteurized at 80°C for 5 min and bottled for further analysis. Triplicate sample analyses were carried out on all fractions after 0, 14, 28, 42 and 56 days of ambient storage (28.5±2°C).

Antioxidant analysis

Determination of ascorbic acid content (AA)

2, 6 dichlorophenolindophenol (DCPIP) assay with spectrophotometric analysis was used to determine ascorbic acid concentration in the samples according to a method described by Bungau et al. (2011) with modifications. Samples were diluted in 0.1 M citric acid and 0.1 M sodium citrate buffers at ratio 1:40 and 1:20. The spectrophotometer (T70 UV-VIS spectrophotometer, PG instruments, Alma Park, UK) absorbance was read at 520 nm. Two millilitres of DCPIP was added to 10 ml of each sample and the tube immediately capped. Absorbance was read within 5 min of adding DCPIP solution. Values for AA were expressed as mg/ml of sample.

Determination of total flavonoid content (TFC)

Aluminum chloride colorimetric method (AlCl₃) was used for the determination of the TFC of samples. About 1.5 ml of each sample was mixed with 5 ml distilled H₂O and 0.3 ml 5% NaNO₂ and 1.5 ml of 2% methanolic AlCl₃ solution was added after 5 min. Double distilled water (ddH₂O) was used instead of sample as blank. Two millilitre 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 read at 367 nm after 10 min incubation period. Total flavonoid contents were calculated using a standard calibration curve prepared for quercetin and expressed as mg quercetin/100 mL of sample (Jagadish et al., 2009).

Determination of total phenolic compounds (TPC)

The Folin–Ciocalteau method was used to quantify the total phenolic compounds by spectrophotometry. About 0.5 ml of sample was introduced into test tubes followed by 2.5 ml of 10% Folin Ciocalteau reagent (Sigma Chemical, St. Louis, Missouri, USA) 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) (Chan et al., 2009).

Determination of ferric reducing antioxidant power (FRAP)

Reducing power was determined using the method of Benzie and Strain (1999) as modified by Adeboyejo et al. (2016). FRAP reagent was prepared by mixing 5 ml of 2,4,6-tris (2-pyridyl)-1,3,5-triazine (TPTZ) solution (10 mM) in 40 mM hydrochloric acid solution with 5 ml FeCl₃·6H₂O solution (20 mM) and 50 mM acetate buffer solution (0.3 M, pH 3.6) and incubated at 37°C after mixing. Extract 150 µl was mixed with 2850 µl of FRAP reagent for 30 min under dark conditions. The absorbance at 593 nm of 200 µl of the mixture was determined (T70 UV-VIS spectrophotometer, PG instruments, Alma Park, UK). FRAP values were compared with quercetin (µmol/ml).
**DPPH Radical-scavenging Activity**

Free radical scavenging activities of samples were determined using the 1, 1-diphenyl-1, 2-picrylhydrazyl (DPPH) method by Blois (1958) and Botchway et al. (2007) with modifications. A solution of 0.002% µM DPPH was prepared by dissolving 0.002 g of DPPH in 80% ethanol. 400 µl of sample was added to 7.6 ml of 0.002% DPPH and left in the dark for 30 min. Concentrations of 0.2, 0.1, 0.05, 0.025, 0.020 and 0.01 mg/ml gallic acid were used to plot the standard curve. The reduction ability of DPPH radicals was determined by the decrease in its absorbance at 517 nm induced by antioxidants after 30 min incubation in the dark. Ethanol was employed as a blank and absorbance read. Activities of test samples were determined as percentage reduction or inhibition of DPPH.

**Determination of total antioxidant activity (TAC)**

Total antioxidant activity of samples were determined by the method of Prieto et al. (1999). 0.1 ml sample (100 µg/ml) was added to 1.9 ml of reagent solution (0.6 M H₂SO₄, 28 mM sodium phosphate and 4 mM ammonium molybdate). The blank solution contained only 2 mL of reagent solution. The absorbance was measured at 695 nm after 60 min. Ascorbic acid was used as standard and total antioxidant capacity was expressed as milligrams of ascorbic acid equivalents (AAE) per 100 ml of sample.

**Anti-nutritional factors assay**

**Determination of tannin**

Tannin was quantitatively determined as reported in the manual of food quality control (AOAC, 1984). 0.5 ml of each sample was mixed with 10 ml of 80% ethanol. This was shaken and allowed to stand for 1 h and 1 ml of extract was pipetted into another test tube. 5 ml distilled water, 2 drops of FeCl₂ in 0.1M HCl was added, shaken and 4 drops of potassium ferrocyanide was added. Absorbance of mixture was read at 620 nm.

**Determination of saponin**

Saponin content was determined as described by Brunner (1984).

**Oxalate content determination**

Oxalate content was determined according to the method described by Oke (1986).

**Determination of phytate**

Phytate analysis was done following a modified procedure of Sivakumaran et al. (2017). 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 D₂O) while constantly shaking at 25°C for 3 h. All extracts were filtered and phytate content determined at 640nm in a spectrophotometer. Amount of phytic acid was calculated from organic phosphorus.

**Statistical analysis**

Data obtained were analysed using Statistical Package for Social Sciences (SPSS) version 21. One-way ANOVA was used to determine the means expressed as mean ± standard deviation (SD) followed by Duncan post-hoc mean test. Test of significance was done at 5% probability level (P<0.05).

**RESULTS AND DISCUSSION**

**Antioxidant assay**

**Ascorbic acid content (AA)**

Data obtained for fresh and stored beverages from ASA fractions were summarized in Figure 1. Evidently, peel fraction contained significantly higher vitamin C level (0.53 mg/ml) than pulp (0.17 mg/ml) and seed coat (0.35 mg/ml) fractions. A steady, rapid (P<0.05) loss of vitamin C was observed in the pulp juice while peel drink retained over 45% of its initial vitamin C content after 56 days of storage. Del Caro et al. (2004) reported similar loss of vitamin C in stored citrus fruit juice while variations have been reported by methods of extraction and maturity stage of fruits (Hernández et al., 2005). However, ascorbic acid content was stabilized by sonication and thermonicitation treatments during storage (Aguilar et al., 2017). Intense irreversible oxidation of L-ascorbic acid, the biologically active form of vitamin C into diketogluconic acid in the presence of air may explain loss of vitamin C in samples. Previous studies showed that free radicals are largely generated in fruit juices produced under aerobic systems, causing oxidation of vitamin C, which thus may explain the result obtained in this study.

**Flavonoid**

Mean concentrations of flavonoids obtained for ASA peel, pulp and seed coat drinks during storage are shown in Figure 2. Total flavonoid content was highest in pulp sample before storage (6.44 mg/100 ml) but significantly decreased (P<0.05) by 73.3, 84.6 and 93.3% after two (1.72 mg/100 ml), four (0.99 mg/100 ml) and six (0.43 mg/100 ml) weeks respectively with slight increase at eight weeks (0.49 mg/100 ml). This indicates a 7.61% retention of original flavonoid content in pulp drink. The seed coat drink retained highest amount at 18.90% while peel drink retained only 10.26% showing a significant variation due to tissue fraction and storage. Hoffmann et al. (2017) similarly observed a trend of total flavonoid degradation during a 3 months storage of *Butia odorata* fruit pulp. 86% loss of initial flavonoid in rowanberry fruit stored at 22°C after 20 weeks of storage has also been reported (Baltacioglu et al., 2011). Ibrahim et al. (2017) reported flavonoid contents of fresh peel, pulp and seed coats fractions of ASA fruit at 17.23, 11.24 and 15.11% respectively. These results are in reverse order to those observed for drinks produced from similar fractions. Degradation of flavonoids in fruit tissues...
and their products may be attributed to activity of enzymes polyphenol oxidase and peroxidase, initiated and sustained by temperature, light, pH and reaction of other components in the product matrix.

**Total phenolic content (TPC)**

The phenol concentrations in pulp sample analyzed at week 0 differed significantly from those of peel and seed drink samples as shown in Figure 3. Values ranged from 3.04 and 3.05 mg/ml in seed and peel drinks to 13.37 mg/ml in pulp juice. A continuous rapid decrease in TPC was observed during the 56 days storage period for pulp juice with only 6.43% of original content retained at the end. Peel and seed drinks however retained 33.12 and 32.24% of phenol after storage duration respectively. This indicates a higher rate of
phenolic content degradation in the pulp fraction beginning from the 2nd week of storage after which 86.46% of phenolic content had been lost. Structural features of major phenolic hydroxyl groups found in plant material may influence the variation of total phenolic content in fruit tissue fractions. The marked reduction of total phenolic content could also result partly from increased oxidation of phenolic substrate to quinone occasioned by high average ambient temperature (26.5±2°C) 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.

**Ferric reducing antioxidant power (FRAP)**

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 (Singh and Rajini, 2004).

The reducing power of the beverages from peel, pulp and seed coat fraction of ASA in storage is presented in Figure 4. The peel fraction exhibited the highest reducing power (22.20 μmol/ml) followed by the seed (11.87 μmol/ml) and pulp (10.90 μmol/ml) fractions. This agrees well with the trend obtained by Prasad et al. (2010) for peel, seed and pulp fractions of Canarium odontophyllum fruit extracts. Guo et al. (2003) earlier confirmed a general trend of similar tissue variation of FRAP values for some fruits. Significant losses of 6.44, 18.11, 51.49 and 63.20% in reducing power of the peel fraction were however observed after 14, 28, 42 and 56 days of ambient storage respectively. Although, initially lower than values recorded for peel fraction (at 53.47 and 49.1% respectively), rate of FRAP loss in seed and pulp fractions were stabilized over the storage period with a total loss of 25.27 and 27.80% compared to 63.20% in the peel fraction after 56 days.

**DPPH radical scavenging activity**

2, 2-di (4-tertocalyphenyl)-1-pircylhydrazyl (DPPH) method is important in quantifying the antioxidant capacity of fruit and vegetable juices or extracts. The DPPH radical scavenging activity of beverages from all fractions of ASA fruit decreased as storage days increased (Figure 5). The peel fraction exhibited the highest scavenging activity at 98.67% compared to pulp (75.30%) and seed coat fractions (76.97%). The seed coat fraction however retained the most activity during storage (71.27%). The scavenging activity loss was in the order pulp>peel>seed fraction.
Figure 4. Changes in ferric reducing antioxidant power of drinks from African star apple peel, pulp and seed coat fractions during storage.

Figure 5. DPPH free radical scavenging activity of drinks from African star apple peel, pulp and seed coat fractions during storage.

at 66.35, 30.4 and 28.73% respectively after 56 day storage. The pulp juice significantly (p<0.005) lost activity of 32.34% between 28 and 42 days alone compared to the loss of only 6.5% between the 14th and 28th days and 11.61% between 42 and 56 days of storage. This implies that the product life cycle of pulp juice till it reach
consumers may be set within 28 days of production during which the product maintains over 77% of its capacity to inhibit the DPPH radical.

**Total antioxidant activity**

Antioxidant activity of beverages made from fruit tissues as measured using phosphomolybdate solution is presented in Figure 6. During storage, a significant (p<0.05) decrease in antioxidant activity was noted. Initial values ranged from 11.85 mg AAE/100 ml (peel) to 9.05 mg AAE/100 ml (pulp) and 8.92 mg AAE/100 ml (seed). Over 56 days of ambient storage, retention of antioxidant activity of pulp juice was significantly higher (22.54%) than peel (17.72%) and seed coat drinks (10.99%), although peel drink had initial higher values. It seems oxidative reactions in the beverages during storage were initiated by light and heat conditions encountered during processing. Reduction in antioxidant activity has been postulated to be due to oxygen induced degradation of vitamin C and other polyphenolic constituents in fruits (Galani et al., 2017). Oxidative stability in fruit beverages may thus be monitored by assessing stability of its vitamin C content during storage. The different tissue fractions, and consequently their products are composed of diverse types and quantity of bioactive compounds which may have influence on their antioxidant activity.

**Anti-nutritional factors**

**Tannin**

Tannins are heat-stable, non-nutritive secondary metabolites and polyphenolic compounds known to have bitter, astringent tastes. Table 1 shows tannin contents of beverages from peel, pulp and seed coat fractions of ASA fruit. Tannin contents increased steadily from 1.79 to 2.77%, 0.17 to 1.23% and 1.83 to 2.61% in peel, pulp and seed coat fractions respectively over the storage period. The pulp juice has a significantly (p<0.05) lower content of tannin than others. Omoboyowa et al. (2016) reported similar values of 127.77 mg tannin in 100 g of fresh C.albidum seed cotyledon as observed for drinks from the seed cotyledon in this study. However, our values are lower than those reported for tropical fruits like banana (3.4 mg/g), apple (8.5 mg/g), guava (20.36 mg/g), pawpaw (10.16 mg/g) and sweet orange (48.16 mg/g) (Onibon et al., 2007). The increase in tannin content on storage could be as a result of increased bioaccessibility of tannins in the chloroplast structure released through mechanical homogenization and pasteurization during processing. Furthermore, irreversible oxidative transformation of proanthocyanidins, 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.
Saponin

Saponin contents of C. albidum beverages are shown in Table 1. Data obtained for all samples are significantly different with peel drink having the highest content at 0.049%. The pulp juice had increased saponin content after 14 days (0.03%) which gradually reduced till end of storage period. After 28 days, all saponin contents stabilized at <0.01%. Ibrahim et al. (2017) established that freeze-dried peel fraction of C. albidum contained higher saponin content (0.41%) than pulp (0.007%) and seed coat (0.09%) fractions, which corresponds well with our findings in this study. 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 (Aletor, 1993).

Oxalate

The oxalate levels in ASA beverages are shown in Table 1. The peel and seed coat drinks had 0.06 mg/g oxalate contents while pulp juice recorded 0.03 mg/g. These values reduced by half after 4 weeks of storage and stabilized till the end of storage period, although these changes were not significant (p<0.05). Oxidative degradation of phenolic compounds as a result of change in pattern of enzyme activity, particularly chloroplast phenolase may be responsible for oxalate degradation in the products under study. Oxalate contents in three varieties of ASA were reported in the range of 0.528-0.538 mg/100 g (Adepoju and Adeniji, 2012). Oxalate nephropathy with acute renal failure has been reported for ingestion of large amount of sour and Averrhoa bilimbi fruit juices (Miah et al., 2018).

Table 1. Variation of anti-nutritional factors in African star apple peel, pulp and seed coat beverages during ambient storage.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Duration of storage (weeks)</th>
<th>Tannin</th>
<th>Phytate</th>
<th>Oxalate</th>
<th>Saponin</th>
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</thead>
<tbody>
<tr>
<td>Peel drink</td>
<td>0</td>
<td>1.79±0.08</td>
<td>0.95±0.01</td>
<td>0.06±0.01</td>
<td>0.05±0.04</td>
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<td></td>
<td>2</td>
<td>1.94±0.05</td>
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<td></td>
<td>4</td>
<td>2.54±0.10</td>
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<td>0.03±0.01</td>
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<tr>
<td></td>
<td>6</td>
<td>2.64±0.09</td>
<td>1.03±0.02</td>
<td>0.03±0.01</td>
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<td></td>
<td>8</td>
<td>2.77±0.04</td>
<td>1.02±0.02</td>
<td>0.03±0.01</td>
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<tr>
<td>Pulp juice</td>
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<td>0.06±0.00</td>
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<td>0.88±0.01</td>
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<td>0.82±0.13</td>
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<td>Seed coat drink</td>
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<td>0.06±0.03</td>
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</table>

*Means ± Standard Deviation superscripted by different alphabets within the same column are significantly different (n=3) (p<0.05).

Phytate

The phytate content in the beverage made from peel increased slightly but insignificantly (p<0.05) from 0.95 mg/100 g at week 0 to 1.02 mg/100 g at week 8 (Table 1). Pulp juice phytate level increased steadily but not significantly after 4 weeks of storage till end of storage period from 0.64 mg/100 g to 0.90 mg/100 g. Phytate content in three varieties of C. albidum was found to range from 0.037 to 0.062 mg/100 g in fresh pulp (Adepoju and Adeniji, 2012), values markedly lower than those observed in this study. These values are significantly lower than 2.88-10.71 mg/g range of phytate content reported for common tropical fruits (Onibon et al., 2007). The slight decrease of phytate levels from 6 weeks of storage is probably related to the capacity of endogenous phytates to be metabolized on incubation. 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.

**Conclusion**

This study reported the influence of storage time on antioxidant and anti-nutritional properties of beverages from peel, pulp and seed coat fractions of African star apple. During the storage period, all studied bioactive components decreased significantly (p<0.05) as storage time increased except tannins and phytates which increased slightly. Highest FRAP and DPPH free radical scavenging activities were retained in the pulp and seed drinks while the peel drink retained highest Vitamin C contents after storage. The results indicated that impact of storage time at ambient conditions on retention of bioactive components of ASA tissue fraction beverages is significant and may be a major deciding factor in setting product shelf life. This study therefore provides invaluable information for guiding bioavailability and oxidative stress biomarker assays for future studies.

**CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

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