

## SPECTROPHOTOMETRIC DETERMINATION OF ASCORBIC ACID (VITAMIN C) AND SELENIUM CONTENT IN SELECTED FRUIT JUICES

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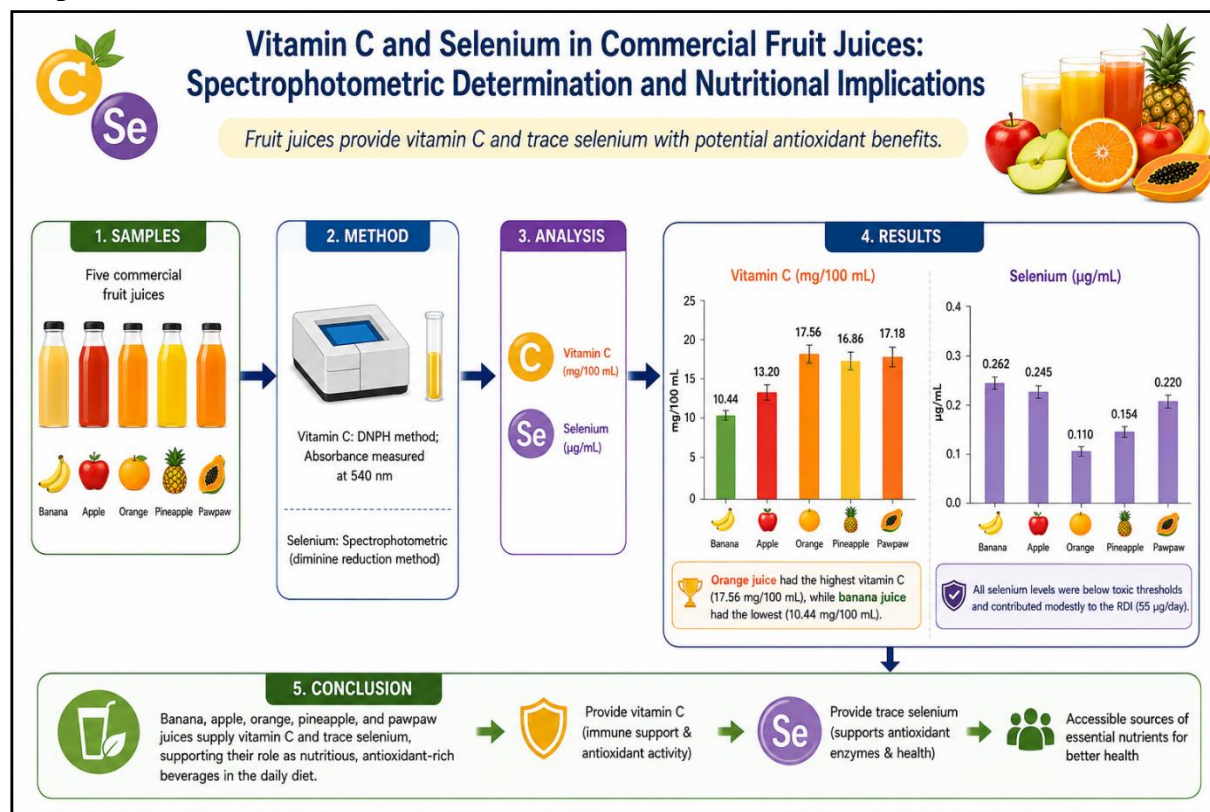
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| Article Information                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | Abstract                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
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| <p>Copyright: © 2026 Agwogie <i>et al.</i> This open-access article is distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.</p> <p><b>Citation:</b> Agwogie, B. A., Okwuenu P. C, Etoh P. A, Okwuenu F. O, Nnajiofor T, &amp; Ekere N. R. (2026). Spectrophotometric determination of ascorbic acid (vitamin C) and selenium content in selected fruit juices. <i>Journal of Chemistry and Allied Sciences</i>, 2(2), 150-158.</p> <p><b>DOI:</b><br/><a href="https://doi.org/10.60787/jcas.vol2no2.73">https://doi.org/10.60787/jcas.vol2no2.73</a></p> <p>The Official Publication of the Tropical Research and Allied Network (TRANet), Department of Chemistry, <i>Federal University of Technology, Minna</i>.</p> | <p>A spectrophotometric method was employed to quantify the ascorbic acid (vitamin C) and selenium content in selected fruit juices. Five fruit juice samples—banana, apple, orange, pineapple, and pawpaw—were analyzed. Vitamin C concentrations were determined by measuring absorbance at 540 nm using a UV–Visible spectrophotometer (Model 1601, Shimadzu, Japan). The results revealed that orange juice contained the highest vitamin C content (<math>17.56 \pm 0.21</math> mg/100 mL), followed closely by pawpaw (<math>17.18 \pm 0.63</math> mg/100 mL) and pineapple (<math>16.86 \pm 0.46</math> mg/100 mL), whereas banana juice had the lowest level (<math>10.44 \pm 0.29</math> mg/100 mL). Selenium concentrations were measured in the same samples, yielding values of <math>0.262 \pm 0.009</math>, <math>0.245 \pm 0.013</math>, <math>0.110 \pm 0.016</math>, <math>0.154 \pm 0.007</math>, and <math>0.220 \pm 0.019</math> µg/mL for banana, apple, orange, pineapple, and pawpaw juices, respectively. All selenium levels were below toxic thresholds and contributed modestly to the recommended daily intake for adults (55 µg/day). The variation in vitamin C and selenium content among the samples may reflect differences in fruit species, ripeness, and post-harvest handling. The findings suggest that these fruit juices provide dietary vitamin C while supplying trace amounts of selenium, supporting their role as nutritious beverages with potential antioxidant benefits. This study underscores the importance of fruit juices as accessible sources of essential nutrients in daily diets.</p> <p><b>Keywords:</b> Spectrophotometric, fruit juice, ascorbic acid, selenium, absorbance</p> |

## Graphical Abstract



## 1.0 INTRODUCTION

Vitamins are organic compounds needed in small quantities for normal physiological functions, metabolism, and maintenance of health. Vitamins are classified into fat-soluble (A, D, E, and K) and water-soluble (B-complex and C) vitamins. Unlike fat-soluble vitamins, water-soluble vitamins are not stored extensively in the body and therefore require regular dietary replenishment [1–3]. Among the water-soluble vitamins, ascorbic acid also known as vitamin C is one of the most important antioxidants, contributing to immune function, collagen synthesis, wound healing, and protection against oxidative stress [4,5].

Selenium is a vital trace element that plays an important role in antioxidant defense systems, thyroid hormone metabolism, and immune function. It is a constituent of several selenoproteins, including glutathione peroxidase, which protects cells against oxidative damage [6,7]. Although selenium is required in trace amounts, however, too much intake can lead to toxicity, making its monitoring in foods and beverages, including fruit juices important. The recommended dietary intake and the narrow margin between selenium deficiency and toxicity highlight the need for accurate determination of selenium levels in commonly consumed fruit juices[8].

Fruits are natural and abundant reservoirs of vitamins, minerals, dietary fibre, and bioactive compounds. They contribute significantly to human nutrition and health. They contain natural antioxidants such as vitamin C, vitamin E, carotenoids, flavonoids, and polyphenols, which have been associated with reduced risks of cardiovascular diseases, cancer, and other chronic disorders [9–11]. Fruits such as pawpaw (*Carica papaya*), apple (*Malus domestica*), pineapple (*Ananas comosus*), orange (*Citrus sinensis*), and banana (*Musa spp.*) are among the most frequently consumed fruits worldwide and are commonly processed into juices for daily consumption. Their popularity and nutritional significance make them important targets for quality assessment and nutritional evaluation.

Several analytical techniques can be employed to quantify ascorbic acid and selenium in food and drinks. These analytical techniques include titrimetric, spectrophotometric, electrochemical, atomic absorption spectrometric, inductively coupled plasma-based, and chromatographic methods [12–16]. The titrimetric methods are reported to be simple and cost-effective and also very suitable for organic compounds such as sugars and vitamins. The High Performance Liquid Chromatography (HPLC) offers superior sensitivity, selectivity, and precision [17].

Spectrophotometric methods remain attractive for selenium determination because of their simplicity, affordability, sensitivity to low concentration, and suitability for routine analysis, particularly [18].

Despite the availability of numerous studies on vitamin C or selenium contents of fruits and fruit products, most investigations have focused on either vitamin C or selenium independently [16-19], with limited studies evaluating both nutrients simultaneously in commonly consumed fresh fruit juices [20]. Furthermore, comparative information on the levels of ascorbic acid and selenium in locally available fruit juices remains scarce, particularly for fruits that are routinely consumed as both fresh produce and processed juices. This knowledge gap limits the availability of comprehensive nutritional data needed for dietary assessment and public health recommendations.

Therefore, this study was aimed at determining the concentrations of ascorbic acid and selenium in selected fruit juices obtained from pawpaw, apple, pineapple, orange, and banana using suitable analytical methods. It also provides comparative data on the nutritional quality of the analyzed fruit juices and contribute to the growing database on micronutrient composition of fruit-derived beverages.

## 2.0 MATERIALS AND METHODS

### 2.1 Materials and Chemicals

The materials and chemicals used in this study were as follows: Volumetric flasks (100 mL and 50 mL), micropipettes (1.0 mL), conical flasks, filter paper, beakers, stirring rod, analytical weighing balance, UV-Visible spectrophotometer (Model 1601, Shimadzu, Japan), ascorbic acid (vitamin C) – pure standard,  $\geq 98\%$  purity, chromium(VI) solution ( $0.01 \text{ mol L}^{-1}$ ), sulphuric acid ( $\text{H}_2\text{SO}_4$ ) ( $1.0 \text{ mol L}^{-1}$ ), diphenylcarbazide (DPC) solution ( $0.25\% \text{ w/v}$ ), selenium dioxide ( $\text{SeO}_2$ ) –  $\geq 98\%$  purity, nitric acid ( $\text{HNO}_3$ ), concentrated hydrochloric acid ( $\text{HCl}$ ), 2,4-Dinitrophenylhydrazine–N-(1-naphthyl)ethylenediamine (DNPH–NEDA) reagent mixture, and distilled water. All chemicals were of analytical grade ( $\geq 98\%$ ). They were purchased from Sigma-Aldrich, Germany.

### 2.2 Sample Collection

Five fruit samples (banana, apple, orange, pineapple, and pawpaw) were sourced from the commercial fruit farm located in Umunedu, Ika North East Local Government Area, Delta State, Nigeria. The samples

were carefully transported to the laboratory using clean polyethylene bags to prevent contamination prior to analysis.

### 2.3 Extraction of Fruit Juice from Samples

The fruit samples (banana, apple, orange, pineapple, and pawpaw) were washed thoroughly with distilled water to remove dust particles. The outer coverings of the fruits were peeled to obtain the fresh mesocarp. The mesocarp of each fruit sample was then processed using a manual fruit juicer to extract the juice. The extracted juice from each fruit was collected in clean 20 mL plastic bottles and stored in a refrigerator at  $4^\circ\text{C}$  for further analysis.

### 2.4 Ascorbic Acid Determination

#### 2.4.1 Preparation of Ascorbic Acid Standard Solution and Sample Analysis

The ascorbic acid standard solution was prepared according to the Centers for Disease Control and Prevention laboratory procedure manual for Vitamin C (ascorbic acid) [21], with slight modifications adapted from Andaririt *et al.*, [22].

Stock solution of ascorbic acid ( $1000 \text{ ppm}$ ) was prepared by accurately weighing  $0.100 \text{ g}$  of pure ascorbic acid into a  $100 \text{ mL}$  volumetric flask, dissolving it in distilled water, and making up to the mark. Working standard solutions ( $1\text{--}5 \text{ ppm}$ ) were prepared by serial dilution of the stock solution. An aliquot ( $1.0 \text{ mL}$ ) of each working standard was transferred into a  $50 \text{ mL}$  volumetric flask. To each flask,  $0.8 \text{ mL}$  of  $0.01 \text{ mol L}^{-1}$  Chromium (VI) solution and  $1.0 \text{ mL}$  of  $1.0 \text{ mol L}^{-1}$  sulphuric acid were added. The mixture was allowed to stand for 10 minutes to enable reduction of  $\text{Cr(VI)}$  by ascorbic acid. Subsequently,  $1.0 \text{ mL}$  of  $0.25\%$  diphenylcarbazide (DPC) solution (usually dissolved in acetone or ethanol and sulfuric acid) was added, and the solution was made up to the mark with distilled water, the remaining  $\text{Cr(VI)}$  will immediately react with DPC to form a red-violet complex. The absorbance of the resulting coloured complex was measured at  $540 \text{ nm}$  against a reagent blank prepared in the same manner but without ascorbic acid. A calibration curve was constructed using blank-corrected absorbance values. The Fruit juice samples were filtered so as to avoid possible interference from other reducing agent in fruit juice and an aliquot ( $1.0 \text{ mL}$ ) was diluted to  $50 \text{ mL}$  with distilled water. A  $1.0 \text{ mL}$  portion of this solution was treated following the procedure described for the standard solutions. The absorbance was measured at  $540 \text{ nm}$  using a UV–Visible spectrophotometer (Model 1601, Shimadzu, Japan). All Vitamin C

determinations were performed in triplicate ( $n = 3$ ), and data were reported as mean  $\pm$  standard deviation.

## 2.5 Selenium Determination

### 2.5.1 Preparation of Selenium Standard Solutions

A 100 ppm selenium stock solution was prepared by dissolving 0.014 g of selenium dioxide ( $\text{SeO}_2$ ) in distilled water, taking into account its purity (98%). Working standard solutions (1–5 ppm) were prepared by diluting 1, 2, 3, 4, and 5 mL of the stock solution to 100 mL with distilled water acidified with 1.5 mL of nitric acid. An aliquot (1.0 mL) of each standard solution was transferred into a 10 mL volumetric flask. To each flask, 5 mL of concentrated hydrochloric acid and 2 mL of 2,4-dinitrophenylhydrazine-N-(1-naphthyl)ethylenediamine (DNPH–NEDA) reagent mixture were added. The mixture was allowed to stand for 10 minutes to allow colour development, after which it was diluted to the mark with distilled water.

The absorbance of the resulting coloured solution was measured at 520 nm using a UV-Visible spectrophotometer (Model 1601, Shimadzu, Japan), with a reagent blank prepared in the same manner but without selenium.

### 2.6 Digestion of Fruit Juice Samples and Analysis

The fruit juice samples were digested using the method as described by Subedi *et al.* [18], with slight modifications. Each fruit juice sample (10 mL) was transferred into a 50 mL conical flask. A mixture of concentrated nitric acid (10 mL) and perchloric acid (5 mL) was added to each sample. The mixtures were heated in a perchloric acid-compatible fume hood until digestion was complete. Complete digestion is indicated by the appearance of dense white fumes and a clear solution. The digested samples were allowed to cool and, if insoluble residues were present, filtered through Whatman No. 42 filter paper before being diluted to 50 mL with distilled water. An aliquot (1.0 mL) of the digested sample was transferred into a test tube and treated according to the procedure described for the standard solutions. The mixtures were allowed to react for 10 minutes to ensure complete colour development, and then diluted to 10 mL with distilled water.

The absorbance of the resulting solution was measured in triplicate ( $n = 3$ ) at 520 nm against a reagent blank using a UV–Visible spectrophotometer (Shimadzu UV-1601, Japan). Selenium concentrations were determined from the calibration curve using the equation:

$$x = \frac{y-c}{m} \quad (1)$$

Where:

$x$  = concentration of selenium in the sample ( $\mu\text{g/mL}$ )

$y$  = absorbance of the sample

$m$  = slope of the calibration curve

$c$  = intercept of the calibration curve

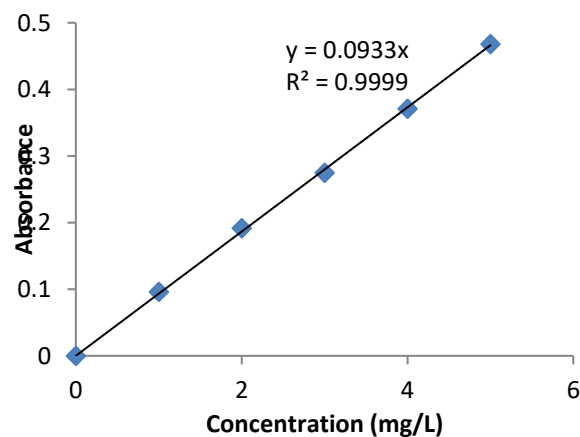
## 3.0 RESULTS AND DISCUSSION

### 3.1 Absorbance of Ascorbic Acid Standard

The absorbance values of the ascorbic acid standards at 540 nm are presented in Table 1. A linear relationship between absorbance and concentration was observed, as shown in Figure 1. The high linearity of the calibration curve ( $R^2 \approx 0.999$ ) indicates that the analytical method employed in this study is suitable for quantifying ascorbic acid in fruit juice samples.

**Table 1:** Absorbance of Ascorbic Acid Standards

| Concentration (mg/L) | Absorbance |
|----------------------|------------|
| 1.0                  | 0.096      |
| 2.0                  | 0.192      |
| 3.0                  | 0.275      |
| 4.0                  | 0.371      |
| 5.0                  | 0.468      |



**Figure 1:** Calibration curve of Ascorbic Acid Standards at 540nm

### 3.2 Concentration of Ascorbic Acid in Fruit Juice Samples

The concentrations of ascorbic acid in the fruit juice samples (banana, apple, orange, pineapple, and pawpaw) are shown in Table 2. Among the fruits juice samples analyzed, Orange ( $17.56 \pm 0.21$  mg/100 mL) has the highest ascorbic acid content, closely followed by pawpaw ( $17.18 \pm 0.63$  mg/100 mL) and Pineapple ( $16.86 \pm 0.46$  mg/100 mL). Banana



contained the lowest concentration at  $10.44 \pm 0.29$  mg/100 mL.

**Table 2:** Concentration of ascorbic acid in fruit juice samples

| Sample    | Abs   | Conc (mg/100 mL) |
|-----------|-------|------------------|
| Banana    | 0.194 | $10.44 \pm 0.29$ |
| Apple     | 0.293 | $15.31 \pm 0.56$ |
| Orange    | 0.327 | $17.56 \pm 0.21$ |
| Pineapple | 0.322 | $16.86 \pm 0.46$ |
| Pawpaw    | 0.324 | $17.18 \pm 0.63$ |

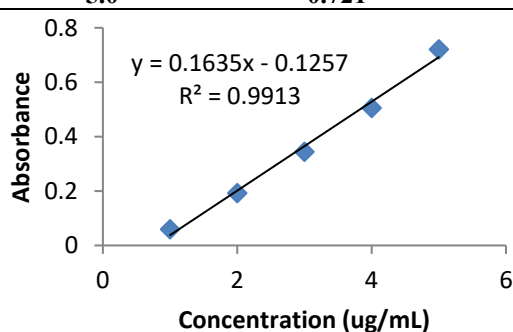
The concentration variation in ascorbic acid among the selected fruits juice samples may be attributed to differences in species, ripeness, and post-harvest handling by the farmers [19]. The results indicate that all fruit juice samples analyzed are good sources of vitamin C, with orange and pawpaw having the higher levels of vitamin C suitable for meeting daily nutritional requirements.

### 3.3 Absorbance of Selenium in Standards

The absorbance of selenium standards at 520 nm is presented in Table 3. The calibration curve for selenium (Figure 2) was constructed by plotting absorbance against standard concentrations (1–5 ppm). The curve exhibits excellent linearity, with a correlation coefficient of  $R^2 \approx 0.999$ , indicating that the method is highly suitable for the quantitative determination of selenium in fruit juice samples.

**Table 3:** Absorbance of Selenium in Standards

| Conc (µg/mL) | Abs Reading |
|--------------|-------------|
| 1.0          | 0.060       |
| 2.0          | 0.193       |
| 3.0          | 0.344       |
| 4.0          | 0.506       |
| 5.0          | 0.721       |



**Figure 2:** Selenium Standard Curve

### 3.4 Concentration of Selenium in Fruit Juice Samples

The concentrations of selenium in the fruit juice samples are presented in Table 4. Banana juice ( $0.262 \pm 0.001$  µg/mL) and apple juice ( $0.245 \pm 0.013$  µg/mL) exhibited the highest selenium levels, while

orange juice showed the lowest concentration ( $0.110 \pm 0.016$  µg/mL). Selenium was detected in all samples, although at low concentrations. When compared with dietary exposure limits (Table 5), the observed levels indicate no evidence of harmful contamination in the analyzed juices, suggesting that they contribute only modestly to selenium intake.

Currently, no specific maximum permissible limits for selenium in fruit juices have been established by major regulatory authorities, including the Codex Alimentarius Commission, US Food and Drug Administration (US FDA), or European Commission food standards [23]. Therefore, selenium levels in beverages are commonly assessed using general contaminant and dietary exposure benchmarks [23, 24]. In a related study, the WHO guideline value for selenium in drinking water (40 µg/L) and the FAO/WHO provisional tolerable upper intake level (UL) of 400 µg/day for adults were used as reference standards for exposure assessment [25, 26].

To evaluate realistic dietary exposure, selenium intake was estimated based on a typical consumption volume of 250 mL/day. The calculated intakes for the juices remain below the FAO/WHO tolerable upper intake level and are within or close to the recommended dietary allowance for adults (approximately 55 µg/day) [25, 26]. These findings indicate that consumption of the analyzed fruit juices is unlikely to pose a toxicological risk while potentially contributing moderately to daily selenium intake.

**Table 4:** Concentration of Selenium in fruit Samples

| Samples   | Concentration (µg/mL) |
|-----------|-----------------------|
| Banana    | $0.262 \pm 0.009$     |
| Apple     | $0.245 \pm 0.013$     |
| Orange    | $0.110 \pm 0.016$     |
| Pineapple | $0.154 \pm 0.007$     |
| Pawpaw    | $0.220 \pm 0.019$     |

**Table 5:** Recommended Dietary Allowance for Selenium (µg/mL) [28].

| Age         | Male | Female | Pregnancy | Lactation |
|-------------|------|--------|-----------|-----------|
| 0–6 months  | 15 * | 15 *   | -         | -         |
| 7–12 months | 20 * | 20 *   | -         | -         |
| 1–3 years   | 20   | 20     | -         | -         |
| 4–8 years   | 30   | 30     | -         | -         |
| 9–13 years  | 40   | 40     | -         | -         |

|                        |    |    |    |           |
|------------------------|----|----|----|-----------|
| <b>14–18<br/>years</b> | 55 | 55 | 60 | <b>70</b> |
| <b>19–50<br/>years</b> | 55 | 55 | 60 | <b>70</b> |
| <b>51+<br/>years</b>   | 55 | 55 | -  | -         |

Although selenium levels in the analyzed fruit juices were below the established dietary thresholds of 40 µg/L and 400 µg/day for adults [25,26], their presence indicates that these fruit juice can contribute modestly to dietary selenium intake. Selenium is an essential trace element involved in antioxidant defense through its incorporation into selenoproteins such as glutathione peroxidase, as well as in thyroid hormone metabolism and immune regulation [27]. The concentrations observed are consistent with reports that fruits generally contain low selenium levels compared with cereals, nuts, and animal-derived foods, reflecting their limited capacity for selenium accumulation [28,29–31].

Variation in selenium content among the juices may be attributed primarily to differences in soil selenium availability and bioavailability, which are the major determinants of plant uptake [32]. Additional environmental factors, including soil pH, organic matter content, rainfall, irrigation practices, and climatic conditions, also influence selenium mobility and absorption. Furthermore, genetic differences among fruit species and cultivars affect selenium uptake and translocation efficiency [32]. Agricultural inputs and soil management practices may further contribute to the observed variability [20,32], while post-harvest processing and juice formulation can introduce dilution effects during extraction and blending. Although the measured concentrations do not pose a health risk, excessive selenium intake is associated with selenosis, characterized by symptoms such as hair and nail brittleness, gastrointestinal disturbances, fatigue, and neurological effects [24,33].

Ascorbic acid concentrations in the fruit juices ranged from 10.4 to 17.6 mg/100 mL, with banana juice exhibiting the lowest level and orange juice the highest. These values are lower than those reported by Oyeyiola and Adetunde [34], but are comparable to findings from other studies, underscoring the variability of vitamin C content in fruit-based products [34,35]. Such variability is strongly influenced by biological, environmental, and processing conditions. Fruit maturity is particularly important, as ascorbic acid may accumulate or degrade during ripening depending on species and storage duration, with overripe fruits typically

showing reduced levels due to oxidative breakdown [35].

Environmental conditions during cultivation—including sunlight exposure, temperature, rainfall, soil fertility, and water availability—also affect ascorbic acid biosynthesis and accumulation [34, 35]. In addition, genetic differences among cultivars contribute to significant inter- and intra-species variation [36]. Post-harvest handling and processing represent further critical factors, as ascorbic acid is highly susceptible to oxidation induced by oxygen, light, heat, and metal ions [37]. Delays between harvesting, processing, and analysis, as well as storage conditions, can therefore result in substantial nutrient losses [37]. Processing steps such as peeling, cutting, blending, filtration, and pasteurization may further accelerate degradation, as documented in previous studies on fruit juice quality [36, 37].

Despite these reductions, the analyzed fruit juices remain valuable dietary sources of antioxidants and contribute to daily vitamin C intake. Ascorbic acid plays key roles in immune function, collagen synthesis, wound healing, iron absorption, and protection against oxidative stress [38]. The increasing consumption of fruit juices, driven by urbanization and health awareness, highlights their nutritional importance as convenient functional beverages [39]. However, their nutritional quality depends strongly on fruit type, cultivation conditions, maturity stage, and processing and storage practices. Therefore, optimizing post-harvest handling and processing conditions is essential to preserve nutrient content and enhance the health benefits of fruit juices [40]. Finally, the strong linearity observed in the calibration curves confirms the reliability and suitability of the analytical methods employed for selenium and ascorbic acid determination, thereby supporting the accuracy and validity of the results obtained in this study.

#### 4.0 CONCLUSION

The spectrophotometric method employed in this study was simple, reliable, and sufficiently sensitive for the determination of ascorbic acid and selenium in selected fruit juice samples. The ascorbic acid contents obtained were generally below recommended dietary intake levels, suggesting that consumption of these fruit juices alone may not be adequate to meet daily vitamin C requirements. Selenium was detected in all samples at low concentrations and contributed minimally to estimated dietary intake. Based on typical consumption patterns, the observed levels indicate low dietary contribution from selenium in the analyzed fruit juices.

Overall, the results demonstrate that the studied fruit juices contain measurable amounts of vitamin C and trace levels of selenium, indicating a modest contribution to dietary intake of these nutrients. Future studies should consider larger sample sizes and the application of advanced confirmatory techniques, including ICP-MS and AAS, to further validate and strengthen the reliability of these findings.

### Authors' Declaration

The authors declare that this work is original, has not been published elsewhere, and is not under consideration by any other journal. The authors accept full responsibility for any claims related to its content.

### Conflict of Interest Statement

The authors declare no conflict of interest.

## Data Availability Statement

The data supporting the findings of this study are available upon reasonable request.

## Authors' Contribution

All authors contributed to the literature search, developed the conceptual framework, data organization, and manuscript drafting. All authors revised the manuscript for intellectual content, validated data, and coordinated the writing process. All authors approved the final version.

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