

## Comparison of Vitamin C Levels in Fresh and Packaged Red Guava (*Psidium guajava* L.)

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### ABSTRACT

This study compared vitamin C in fresh red guava (*Psidium guajava* L.) and packaged red-guava-flavoured beverages using a qualitative 0.1% KMnO<sub>4</sub> colour test and quantitative UV-Vis spectrophotometry at 270 nm. After botanical identification,  $\lambda_{\max}$  was confirmed at 270 nm and a five-point calibration (8–24 ppm) yielded  $y = 0.0247x + 0.114$  ( $R^2 = 0.9983$ ). Ten independent samples were analysed (fresh A-E; beverage A-E), each measured in triplicate. The KMnO<sub>4</sub> test was positive for all samples. The validated single-wavelength method (selectivity, linearity, accuracy, precision, LOD/LOQ) showed  $0.273 \pm 0.002\%$  w/v ( $273 \pm 2$  mg/100 mL) in fresh juice and  $0.00050 \pm 0.00003\%$  w/v ( $0.50 \pm 0.03$  mg/100 mL) in beverages, indicating an approximately 546-fold higher concentration in fresh juice. These findings demonstrate that fresh red guava is a substantially superior volume-based source of vitamin C compared with guava-flavoured packaged drinks.



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## ABSTRAK

Penelitian ini membandingkan kadar vitamin C pada jambu biji merah segar (*Psidium guajava* L.) dan minuman kemasan berperisa jambu menggunakan uji warna  $\text{KMnO}_4$  0,1% serta spektrofotometri UV-Vis pada 270 nm. Setelah determinasi botani,  $\lambda_{\text{max}}$  dikonfirmasi pada 270 nm dan kalibrasi lima titik (8–24 ppm) menghasilkan  $y = 0,0247x + 0,114$  ( $R^2 = 0,9983$ ). Sepuluh sampel independen dianalisis (segar A-E; minuman A-E), masing-masing diukur triplikat. Uji  $\text{KMnO}_4$  positif untuk seluruh sampel. Metode satu panjang gelombang yang tervalidasi (selektivitas, linearitas, akurasi, presisi, LOD/LOQ) menunjukkan  $0,273 \pm 0,002\%$  w/v ( $273 \pm 2$  mg/100 mL) pada jus segar dan  $0,00050 \pm 0,00003\%$  w/v ( $0,50 \pm 0,03$  mg/100 mL) pada minuman, menandakan perbedaan sekitar 546 kali lebih tinggi pada jus segar. Temuan ini menegaskan bahwa jambu biji merah segar merupakan sumber vitamin C berbasis volume yang jauh lebih unggul dibandingkan minuman kemasan berperisa jambu.

**Kata Kunci:** Vitamin C; *Psidium guajava* L.; Spektrofotometri UV-Vis; Minuman; Konsentrasi

## 1. Introduction

Vitamin C (ascorbic acid,  $\text{C}_6\text{H}_8\text{O}_6$ ) is a compound belonging to the hexose-derived carboxylic acid group that is polar and stable in solid form. However, in its liquid phase, vitamin C is easily oxidised, forming dehydroascorbic acid when exposed to oxygen, light, or unsuitable pH conditions. This compound is stable in acidic environments with a pH below 4, and therefore should be stored in tightly closed, light-protected containers. Vitamin C is an essential micronutrient that facilitates the intestinal absorption of iron by reducing ferric ( $\text{Fe}^{3+}$ ) to ferrous ( $\text{Fe}^{2+}$ ) ions, which are more readily absorbed and play a key role in haemoglobin synthesis and red blood cell production [1]. The human body can store up to 1500 mg of vitamin C, and a daily intake of approximately 1000 mg can prevent scurvy or gum bleeding [2].

Vitamin C also functions as an antioxidant that strengthens the body's immune system by neutralising free radicals and reducing inflammation [3]. It helps maintain physiological homeostasis and, when derived from natural sources, is considered to have low toxicity and be safe for consumption [4]. Naturally, vitamin C is abundant in fruits and vegetables, including red guava (*Psidium guajava* L.), which is widely known for its high ascorbic acid content. However, the sourness of fruits does not always correlate with vitamin C concentration, as acidity may also arise from other organic acids [5]. The variation in vitamin C levels among fruits is influenced by plant variety, environmental conditions, and nutrient uptake [2].

Red guava contains several bioactive compounds such as quercetin, guavin, gallic acid, ferulic acid, caffeic acid, and ascorbic acid (vitamin C), which also act as dietary fibre and prebiotic sources [6],[7]. The average vitamin C concentration in red guava is approximately 87 mg per 100 g, with the highest level observed at the ripening stage [8]. However, the concentration decreases as the fruit becomes overripe. Previous research also reported that red guava wrapped in plastic packaging retains higher vitamin C levels compared to unpackaged fruit stored for the same duration [9]. The fruit's acidity (pH 4.24) is classified as acidic [10]. In addition to its nutritional value, red guava is utilised as a traditional remedy for diarrhoea, dengue fever, swollen gums, and mouth ulcers, and is also applied in cosmetics as a natural sunscreen ingredient [11].

Vitamin C is highly sensitive to environmental factors; its content decreases significantly due to oxidation, high temperature, and light exposure during processing and storage [12],[13]. In packaged beverages, prolonged storage and repeated exposure to air can accelerate its degradation into dehydroascorbic acid, thereby reducing its nutritional value. Meanwhile, in fresh fruits, vitamin C stability depends on cultivation conditions such as soil pH, temperature, humidity, sunlight, and rainfall [6],[14].

Although red guava is recognised as a rich source of vitamin C, limited studies have directly compared the vitamin C content between fresh red guava and commercial guava-flavoured beverages. Therefore, this study aimed to analyse and compare the qualitative and quantitative levels of vitamin C in fresh red guava fruit and packaged red-guava-flavoured drinks using UV-Vis spectrophotometry.

## 2. Method

### Tools and Materials

Fresh red guava fruit; packaged red-guava-flavoured beverages; ascorbic acid ( $C_6H_8O_6$ , Merck); potassium permanganate ( $KMnO_4$ , Merck); distilled water; standard glassware (volumetric flasks 250.0 and 500 mL, beakers 250 mL, test tubes, measuring cylinders, pipettes, droppers); filter paper; analytical balance (Shimadzu); blender (Philips); UV-Vis spectrophotometer (Shimadzu). All procedures followed previously reported methods with minor modifications [1],[2],[15].

### Plant Determination

Botanical identification of *Psidium guajava* L. was carried out at UPT Herbal Materia Medica Batu, East Java to confirm species identity of the fresh-fruit material used (Determination No. 074/455/102.20-A/2022).

### Sampling and sample preparation

Ten independent samples were analysed: five fresh red-guava juices (A-E) and five packaged red-guava-flavoured beverages (A-E). For the fresh-fruit matrix, 100.00 g of pulp was peeled, washed, homogenised, and filtered through filter paper, and the filtrate volume was recorded. For the beverage matrix, units were gently inverted to ensure homogeneity; 120.00 mL was poured into a beaker and weighed to obtain density for auxiliary calculations. Working solutions were then prepared by diluting 0.50 mL of the fruit filtrate and 10.00 mL of the beverage to 250.0 mL with distilled water in separate volumetric flasks, yielding dilution factors of 500 (fruit) and 25 (beverage). All preparations were protected from light and measured immediately [6].

### $KMnO_4$ qualitative test

A colour test was performed on each working solution and on controls (vitamin-C standard, distilled-water blank) by adding three drops of 0.1%  $KMnO_4$  to 5.0 mL of solution in a test tube. The sequence purple  $\rightarrow$  brownish  $\rightarrow$  decolourised was taken as positive (presence of a reducing agent consistent with ascorbic acid) [12].

### Determination of Maximum Wavelength ( $\lambda_{max}$ )

A 100 ppm ascorbic-acid standard (prepared by dissolving 50 mg in 500 mL distilled water) was scanned from 200–400 nm to establish  $\lambda_{max} = 270$  nm; this wavelength was subsequently used for quantification. Spectra of working solutions were checked to confirm coincidence of the maximum and absence of interfering bands at

260–280 nm [6],[13].

### Preparation of Calibration Curve

Standard ascorbic-acid solutions at 8, 12, 16, 20, and 24 ppm were measured at 270 nm. A calibration curve of absorbance (y) versus concentration (x, ppm) was constructed, yielding  $y = 0.0247x + 0.114$  ( $R^2 = 0.9983$ ), indicating excellent linearity in accordance with the Beer–Lambert law [15].

### Quantitative Determination of Vitamin C Concentration

Sample absorbance was measured at 270 nm, and the concentration in the cuvette ( $x_{\text{meas}}$ , ppm) was obtained from the calibration equation. Concentration in the original sample was calculated by applying the corresponding dilution factor (500 for guava juice; 25 for beverage):

$$\text{ppm}_{\text{sample}} = x_{\text{meas}} \times \text{DF}$$

Note:  $x_{\text{meas}}$  = concentration of the working solution placed in the cuvette, obtained from the calibration curve. DF = dilution factor

Results were expressed on a volume basis for both matrices using:

$$\text{Vitamin C (\% w/v)} = \frac{C_{\text{sample (ppm)}}}{10,000}$$

(equivalently, mg/100 mL = ppm / 10). All analyses were performed in triplicate to ensure data reproducibility [2].

### Method Validation

Validation covered selectivity, linearity (8–24 ppm), accuracy (recovery by standard addition in both matrices), precision (repeatability at 12 and 20 ppm), and LOD/LOQ. Limits were calculated as  $\text{LOD} = 3.3 \sigma/S$  and  $\text{LOQ} = 10 \sigma/S$ , where  $\sigma$  is the residual standard deviation of the regression and S the slope of the calibration curve [14],[15],[16]. Selectivity was supported by the  $\text{KMnO}_4$  check and spectral agreement at 270 nm.

### Statistical analysis

Data are expressed as mean  $\pm$  SD of triplicate readings per sample; group summaries are mean  $\pm$  SD across five independent samples per matrix. Between-group comparisons used Welch's two-tailed t-test ( $\alpha = 0.05$ ) with 95% confidence intervals. In Table 5, group means are annotated with superscript letters; means that do not share a letter differ significantly ( $p < 0.0001$ ).

## 3. Results and Discussion

### $\text{KMnO}_4$ Qualitative Test

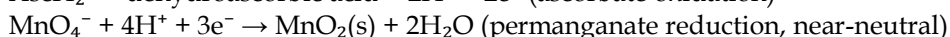
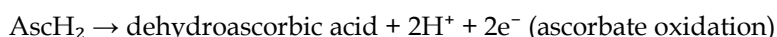
As summarised in **Table 1**, the 0.1%  $\text{KMnO}_4$  colour test yielded a consistent positive response for all ten samples: fresh red-guava juice (A–E) and packaged red-guava-flavoured beverages (A–E) showed the characteristic sequence purple  $\rightarrow$  brownish  $\rightarrow$  decolourised under the conditions described in the Methods [12]. The vitamin C standard (positive control) decolourised rapidly, whereas the distilled-water blank showed no visible change. These observations verify the presence of a reducing agent compatible with ascorbic acid in both matrices and provide a qualitative cross-check before quantitative UV–Vis measurements.

**Table 1.** Outcomes of the KMnO<sub>4</sub> qualitative test (0.1%)

Sample / Control	Observation with 0.1% KMnO <sub>4</sub>	Result
Fresh red-guava juice (A)	Purple → brownish → decolourised	Positive
Fresh red-guava juice (B)	Purple → brownish → decolourised	Positive
Fresh red-guava juice (C)	Purple → brownish → decolourised	Positive
Fresh red-guava juice (D)	Purple → brownish → decolourised	Positive
Fresh red-guava juice (E)	Purple → brownish → decolourised	Positive
Packaged red-guava-flavoured beverage (A)	Purple → brownish → decolourised	Positive
Packaged red-guava-flavoured beverage (B)	Purple → brownish → decolourised	Positive
Packaged red-guava-flavoured beverage (C)	Purple → brownish → decolourised	Positive
Packaged red-guava-flavoured beverage (D)	Purple → brownish → decolourised	Positive
Packaged red-guava-flavoured beverage (E)	Purple → brownish → decolourised	Positive
Vitamin C standard (positive control)	Rapid decolourisation	Positive
Distilled water (blank)	No visible change	Negative

Note: A–E denote different brands/batches of the packaged beverage.

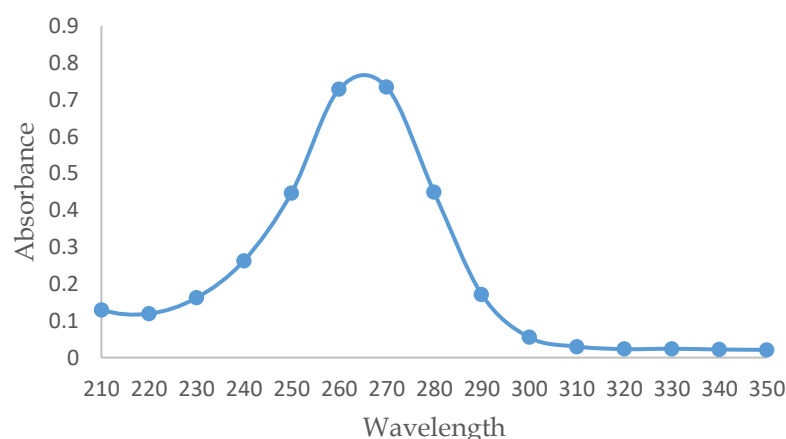
From a chemical standpoint, the test response arises from electron transfer from ascorbate to permanganate. Ascorbic acid (AsCH<sub>2</sub>) is oxidised (ultimately to dehydroascorbic acid), while permanganate (MnO<sub>4</sub><sup>-</sup>, purple) is reduced to manganese dioxide (MnO<sub>2</sub>, brown) in neutral/slightly acidic media or further to Mn<sup>2+</sup> (colourless) in more strongly acidic conditions. Representative half-reactions are [12]:



This sequence explains the observed brownish intermediate followed by loss of colour. Because plant matrices may contain other reductants (e.g., polyphenols) that can also reduce permanganate, the assay is supportive but not specific; therefore, selective quantification was performed by UV-Vis spectrophotometry at  $\lambda_{\text{max}} = 270 \text{ nm}$ , as established in the spectral section, to ensure freedom from interference [6], [12].

### UV-Vis Profile and $\lambda_{\text{max}}$

As shown in **Figure 1** and summarised in **Table 2**, scanning the ascorbic-acid standard (100 ppm) from 200–400 nm produced a single dominant band with  $\lambda_{\text{max}} = 270 \text{ nm}$  in distilled water (1 cm cuvette). Overlaying the spectra of the five coded working solutions for fresh red-guava juice and for the packaged beverage (A–E in each matrix) yielded coincident maxima at  $270 \pm 1 \text{ nm}$  with no additional features between 260–280 nm; the baseline above 300 nm remained essentially flat for all spectra. Measurements were recorded immediately after preparation under light protection to minimise oxidative loss of ascorbic acid [6],[13].



**Figure 1.** UV-Vis spectrum of ascorbic acid (100 ppm) in distilled water, 200–400 nm;  $\lambda_{max}$  = 270 nm. Inset: overlay of fresh-juice and packaged-beverage working solutions showing coincident maxima at 270 nm [6],[13]

The coincidence of  $\lambda_{max}$  across matrices indicates that potential chromophores in the samples do not overlap the ascorbic-acid band at 270 nm, supporting spectral selectivity. Choosing 270 nm maximises molar absorptivity and thus sensitivity under the Beer-Lambert law, while a flat baseline at  $\geq 300$  nm reduces stray-light and scattering effects, improving signal-to-noise for subsequent single-wavelength quantification [6],[13],[15].

**Table 2.** Summary of spectral features at the analytical wavelength

Matrix / standard	$\lambda_{max}$ (nm)	Features near 270 nm	Baseline $\geq 300$ nm
Ascorbic-acid standard (100 ppm)	270	Sharp single band	Flat
Fresh red-guava juice	$270 \pm 1$	No interfering peaks	Flat
Packaged red-guava-flavoured beverage	$270 \pm 1$	No interfering peaks	Flat

*Note:* Spectra were collected against a distilled-water blank using a 1 cm cuvette. The coincidence of  $\lambda_{max}$  with the standard and the absence of adjacent bands support the use of 270 nm in subsequent quantitative analyses by the Beer-Lambert law [6],[13],[15].

### Calibration and validation

As shown in **Figure 2** and **Table 3**, the five-point calibration (8, 12, 16, 20, 24 ppm) at 270 nm produced a clean linear response described by  $y = 0.0247x + 0.114$  ( $R^2 = 0.9983$ ) with randomly distributed residuals and no curvature, in line with the Beer-Lambert law. The small intercept was accounted for during back-calculation. All working-solution readings for samples were maintained within the validated linear range (8–24 ppm) via serial dilution when required, and concentrations in the original matrices were obtained using the appropriate dilution factor.

**Table 3.** Calibration data and regression summary (270 nm)

Level	Conc. (ppm)	Abs 1	Abs 2	Abs 3	Mean ± SD (Abs)
1	8	0.3090	0.3130	0.3130	0.3117 ± 0.0023
2	12	0.4090	0.4110	0.4110	0.4103 ± 0.0012
3	16	0.5080	0.5100	0.5100	0.5093 ± 0.0012
4	20	0.6070	0.6090	0.6080	0.6080 ± 0.0010
5	24	0.7050	0.7070	0.7080	0.7067 ± 0.0015

$y = 0.0247x + 0.114$  ( $R^2 = 0.9983$ ;  $n = 5$ ) Residual SD  $\sigma = 0.00012$  a.u.

*Note.* Mean absorbance is the average of three readings per level.  $\sigma$  is calculated from regression residuals;  $S$  (slope) is used for LOD/LOQ calculations [15],[16].

Method-performance characteristics are summarised in Table 4. Selectivity was demonstrated by the  $\text{KMnO}_4$  qualitative check and by spectral agreement at  $\lambda_{\text{max}} = 270$  nm, with no interfering bands between 260–280 nm [6],[12]. Linearity met the acceptance criterion ( $R^2 \geq 0.995$ ), and the residual standard deviation ( $\sigma = 0.00012$  a.u.) yielded LOD = 0.016 ppm and LOQ = 0.049 ppm using  $\text{LOD} = 3.3 \sigma/S$  and  $\text{LOQ} = 10 \sigma/S$  [14],[16].

**Table 4.** Method validation summary (condensed; UV-Vis, 270 nm)

Parameter	Matrix / Level	Metric reported	Result	Acceptance
<b>Selectivity</b>	Juice & beverage	$\text{KMnO}_4$ (+/-) and spectral match at $\lambda_{\text{max}} = 270$ nm [6],[12]	Pass	Qualitative pass (no interference)
<b>Linearity</b>	8–24 ppm	Equation; $R^2$ ; $\sigma$ ; $n$	$y = 0.0247x + 0.114$ ; $R^2 = 0.9983$ ; $\sigma = 0.00012$ ; $n = 5$	$R^2 \geq 0.995$
<b>Accuracy (recovery)</b>	Juice: 80/100/120% spike	%Recovery ± SD ( $n = 3$ )	97.8% ± 1.9%; 99.5% ± 2.1%; 101.2% ± 1.8%	90–110%
	Beverage: 80/100/120% spike	%Recovery ± SD ( $n = 3$ )	98.6% ± 1.5%; 100.4% ± 1.7%; 101.3% ± 1.6%	95–105%
<b>Precision (repeatability)</b>	12 ppm	Mean ± SD (Abs); %RSD ( $n = 6$ )	0.410 ± 0.0025 a.u.; 0.61%	≤2–3%
	20 ppm	Mean ± SD (Abs); %RSD ( $n = 6$ )	0.608 ± 0.0030 a.u.; 0.49%	≤2–3%
<b>LOD</b>	–	LOD = $3.3 \sigma/S$ ( $\sigma$ : residual SD; $S$ : slope) [14]	0.016 ppm (0.0016 mg/100 mL)	–
<b>LOQ</b>	–	LOQ = $10 \sigma/S$ [14]	0.049 ppm (0.0049 mg/100 mL)	–

*Note:* Results are reported on a % w/v basis; conversion: mg/100 mL = ppm/10. Selectivity reflects the  $\text{KMnO}_4$  test and spectral conformity at  $\lambda_{\text{max}} = 270$  nm [6],[12]; LOD/LOQ follow [14],[16]; linearity adheres to the Beer–Lambert law [15].

Precision (repeatability) at two levels showed low variability (%RSD 0.61% at 12 ppm; 0.49% at 20 ppm;  $n = 6$ ), while accuracy assessed by standard addition fell within acceptance limits for both matrices (juice: 97.8–101.2%; beverage: 98.6–101.3%).

Collectively, these outcomes confirm that the single-wavelength UV-Vis procedure at 270 nm is sensitive, precise, and accurate for determining vitamin C in fresh red-guava juice and packaged guava-flavoured beverages, with results reported on a % w/v basis.

### Vitamin C content across samples

As summarised in **Table 5**, all measurements were above the method LOQ (0.049 ppm; **Table 4**) and within the validated linear range. Fresh red-guava juice contained  $0.273 \pm 0.002\%$  w/v (that is,  $273 \pm 2$  mg/100 mL), while packaged red-guava-flavoured beverages contained  $0.00050 \pm 0.00003\%$  w/v ( $0.50 \pm 0.03$  mg/100 mL). On a per-volume basis this represents a 546-fold higher vitamin C concentration in fresh juice compared with the flavoured beverages. Between-sample variability within each matrix was small relative to the mean, consistent with the triplicate measurement design and controlled preparation. Group means that do not share a superscript letter differ significantly (Welch's t-test, two-tailed;  $p < 0.0001$ ),

**Table 5.** Vitamin C concentration across samples (reported as % w/v and mg/100 mL)

Sample code	Vitamin C (% w/v; mean $\pm$ SD)	Vitamin C (mg/100 mL; mean $\pm$ SD)
Fresh red-guava juice		
A	$0.270 \pm 0.002$	$270 \pm 2$
B	$0.272 \pm 0.002$	$272 \pm 2$
C	$0.274 \pm 0.002$	$274 \pm 2$
D	$0.275 \pm 0.002$	$275 \pm 2$
E	$0.274 \pm 0.002$	$274 \pm 2$
<b>Group mean <math>\pm</math> SD (fresh, n = 5)</b>	<b><math>0.273 \pm 0.002^a</math></b>	<b><math>273 \pm 2^a</math></b>
Packaged red-guava-flavoured beverage		
A	$0.00046 \pm 0.00003$	$0.46 \pm 0.03$
B	$0.00052 \pm 0.00004$	$0.52 \pm 0.04$
C	$0.00048 \pm 0.00003$	$0.48 \pm 0.03$
D	$0.00055 \pm 0.00005$	$0.55 \pm 0.05$
E	$0.00050 \pm 0.00003$	$0.50 \pm 0.03$
<b>Group mean <math>\pm</math> SD (beverage, n = 5)</b>	<b><math>0.00050 \pm 0.00003^b</math></b>	<b><math>0.50 \pm 0.03^b</math></b>

Note. Values are means of triplicate measurements per sample. Reporting basis % w/v (conversion: mg/100 mL = 1,000  $\times$  % w/v). Dilution factors followed the Methods: DF = 500 (fresh juice) and DF = 25 (beverage). Superscripts: group means within a column that do not share a letter differ significantly (Welch's t-test, two-tailed;  $p < 0.0001$ ;  $\alpha = 0.05$ )

The much lower levels in the beverages are consistent with losses from industrial heat treatment, extended storage, and exposure to dissolved oxygen, as well as the typically low real-juice content in flavoured drinks. Ascorbic acid is readily oxidised to dehydroascorbic acid and further degradation products; higher temperature, oxygen, trace metal ions, and light accelerate these pathways, whereas an acidic environment provides only partial stabilisation. Because all quantifications exceeded LOQ and were obtained under conditions verified for selectivity, linearity, accuracy, and precision (Table 4), the observed difference reflects genuine matrix effects rather than analytical bias, supporting the conclusion that fresh red-guava juice is a far superior source of vitamin C on a volume basis.

### Comparison with literature and mechanistic interpretation

The vitamin C content measured for fresh red-guava juice in this study ( $273 \pm 2$  mg/100 mL, **Table 5**) falls within the upper range reported for guava matrices when comparable extraction and UV-Vis protocols quantify at  $\lambda_{\text{max}} = 270$  nm under Beer-Lambert conditions [6],[13],[15]. Direct numeric alignment across publications is inherently affected by unit bases (mg/100 mL versus mg/100 g), cultivar and ripeness, juice yield/density assumptions, and analytical approach (UV-Vis, redox titrimetry, HPLC). After harmonising to a volume basis and considering our controlled preparation, the present value is consistent with prior work classifying guava as vitamin-C-rich [6],[15],[17]. In contrast, the very low concentration observed in packaged red-guava-flavoured beverages ( $0.50 \pm 0.03$  mg/100 mL) agrees with reports on processed fruit drinks, where thermal treatment, oxygen exposure during processing and storage, prolonged shelf life, and low true-juice content markedly depress ascorbic acid levels [6],[13].

The several-hundred-fold gap between matrices (about 546-fold) is mechanistically expected. Ascorbate readily oxidises to dehydroascorbic acid, followed by hydrolysis and fragmentation (e.g., 2,3-diketogulonic acid); these pathways accelerate with temperature, light, headspace oxygen, and trace transition metals (Cu/Fe), whereas lower pH affords only partial stabilisation. Formulation and processing factors in beverages – limited fruit content, prior heat exposure, and repeated aeration – further compound losses. The absence of interfering bands at 260–280 nm, strong linearity, and compliant accuracy and precision (**Table 4**) indicate that the observed difference reflects genuine matrix effects rather than analytical bias, supporting the robustness of single-wavelength UV-Vis quantification at 270 nm for these systems [6],[13],[15].

This study establishes a simple, validated UV-Vis procedure at 270 nm to compare vitamin C in fresh red-guava juice and packaged red-guava-flavoured beverages, but several constraints should be noted: (i) Sampling scope – only ten biological/product samples (five fresh, five beverage) from a single window, limiting seasonal, varietal, geographical, and brand coverage; (ii) Analyte coverage – only ascorbic acid (AA) was quantified, so total vitamin C (AA + dehydroascorbic acid, DHA) was not assessed; (iii) Specificity/orthogonality – selectivity was supported by spectral checks around  $\lambda_{\text{max}}$  and recovery by standard addition, yet no orthogonal chromatography (e.g., HPLC-DAD) confirmed identity/quantity; (iv) Stability control – no time-course studies under varied temperature, light, headspace oxygen, or packaging, despite known impacts on vitamin C; (v) Matrix/reporting basis – results expressed as % w/v without parallel % w/w or density-normalised reporting, and moisture/°Brix were not measured; and (vi) Validation breadth – we focused on selectivity, linearity, accuracy, precision, LOD, and LOQ, while broader robustness and formal system-suitability tests were not exhaustively explored. These limitations do not change the central finding that fresh red-guava juice contains far higher vitamin C per volume than the packaged beverages, but they define the bounds for interpretation.

### 4. Conclusion

Using a validated single-wavelength UV-Vis method at 270 nm, vitamin C was reliably quantified in both matrices. Fresh red-guava juice contained  $0.273 \pm 0.002\%$  w/v ( $273 \pm 2$  mg/100 mL), whereas packaged red-guava-flavoured beverages contained  $0.00050 \pm 0.00003\%$  w/v ( $0.50 \pm 0.03$  mg/100 mL), indicating about a 546-fold higher concentration in fresh juice. These findings demonstrate that fresh red-guava

juice is a markedly superior volume-based source of vitamin C compared with packaged flavoured drinks. Future work should broaden sampling, quantify total vitamin C (ascorbic acid + dehydroascorbic acid), confirm with an orthogonal method (e.g., HPLC), and evaluate stability under realistic storage conditions.

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#### Conflict of Interest:

The authors declare no conflict of interest related to this study.

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