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Determination of vitamin C content in fresh and processed fruits by titrimetric method

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Abstract

Every nutrition label on a carton of orange juice lists a vitamin C value, but few consumers ask how that number was measured or how much of the original ascorbic acid survived pasteurization and storage. Iodometric titration a method old enough to appear in introductory chemistry textbooks remains one of the simplest and cheapest ways to quantify ascorbic acid in food matrices. This research applied the 2, 6-dichlorophenolindophenol (DCPIP) titrimetric method to twelve fruits in five states: fresh, refrigerated (4°C, 7 days), boiled (100°C, 5 min), freshly juiced, and commercially canned. Guava had the highest fresh vitamin C content at 228.4 mg/100 g, followed by kiwi (93.2), lemon (77.4), and strawberry (58.9). Boiling caused the greatest loss an average 45.9% reduction across all fruits while refrigeration preserved 82-92% of the original content. Titrimetric values correlated strongly with parallel HPLC measurements ($R^2 = 0.97$), confirming that the DCPIP method, despite its age, remains fit for purpose in food-quality laboratories with limited instrumentation.

Keywords: Vitamin C, ascorbic acid, titration, DCPIP, fruit, processing, boiling, refrigeration, HPLC, food analysis

Introduction

Ascorbic acid is the most labile of the water-soluble vitamins sensitive to heat, oxygen, light, alkaline pH, and metal ions, all of which accelerate its irreversible oxidation to dehydroascorbic acid and, eventually, to 2,3-diketogulonic acid, which has no vitamin activity^[1]. This fragility means that the vitamin C content printed on a food label may not reflect what is left by the time the product reaches a consumer's plate. The gap between "as harvested" and "as consumed" values can exceed 50% for fruits subjected to thermal processing^[2].

The DCPIP (2, 6-dichlorophenolindophenol) redox titration is one of the oldest and most widely taught methods for ascorbic acid determination. Ascorbic acid reduces the blue dye to its colourless leuco form; the endpoint is the first persistent pink colour^[3]. The method is rapid, requires no expensive equipment, and performs well in simple matrices like fruit juices. Its main weakness is interference from other reducing agents particularly sulfite preservatives and certain sugars which can give falsely high readings^[4]. For routine quality control in developing-country laboratories, however, DCPIP titration often remains the only practical option^[5].

This research measured vitamin C in twelve commonly consumed fruits across five processing conditions using DCPIP titration, validated the results against HPLC as a reference method, and calculated retention percentages to rank processing methods by their impact on ascorbic acid content.

Safety Considerations

DCPIP solution is a mild irritant; gloves were worn during preparation. Metaphosphoric acid (used for extraction) is corrosive at high concentrations; it was handled in a fume hood. No biological hazards were involved. All waste was neutralized and disposed of according to institutional protocols^[6].

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Material and Methods

Material

Work was conducted jointly at Lala Lajpat Rai College of Pharmacy, Meerut, and Vidyasagar University, Midnapore, from February to August 2023. Twelve fruits were purchased from local markets in Meerut: orange, apple, banana, guava, mango, grape, papaya, kiwi, pear, lemon, pineapple, and strawberry. Only ripe, unblemished specimens were selected. L-ascorbic acid standard ($\geq 99\%$, Sigma-Aldrich), DCPIP sodium salt (Merck), metaphosphoric acid (HPO_3 , 33.5%, Loba Chemie), and glacial acetic acid (Rankem) were used as received [7].

Methods

Each fruit was prepared in five states:

- Fresh (analysed within 2 h of purchase),
- Refrigerated at 4°C for 7 days,
- Boiled in water at 100°C for 5 min and cooled,
- Freshly juiced using a domestic blender, and
- Commercially canned equivalents purchased from supermarkets.

For extraction, 10 g of edible portion was homogenized in 50 mL of 3% metaphosphoric acid-acetic acid solution, filtered through Whatman No. 1 paper, and diluted to 100 mL [3]. DCPIP solution (0.025%) was standardized daily against freshly prepared ascorbic acid standard (1 mg/mL in 3% HPO_3). Aliquots (5 mL) of extract were titrated until a persistent pink colour lasted 15 s. Each sample was analysed in triplicate.

HPLC validation used a Shimadzu LC-2040C with a C_{18} column (250×4.6 mm, 5 μm), isocratic elution with 0.1% oxalic acid in water at 1.0 mL/min, detection at 254 nm. Calibration covered 5–200 mg/L ascorbic acid ($R^2 > 0.999$) [8]. Retention (%) was calculated as (processed value / fresh value) $\times 100$.

Statistical Analysis: Two-way ANOVA (fruit $\times$ processing method) with Tukey HSD post-hoc was performed at $p < 0.05$. Pearson correlation compared titrimetric and HPLC values. All statistics used IBM SPSS 28 [9].

Results

Table 1. Vitamin C content (mg/100 g edible portion) by DCPIP titration across five processing states for selected fruits

Fruit	Fresh	Refrigerated	Boiled	Juiced	Canned
Guava	228.4 $\pm$ 6.3	203.7 $\pm$ 5.1	118.6 $\pm$ 4.8	174.3 $\pm$ 5.6	87.7 $\pm$ 3.9
Kiwi	93.2 $\pm$ 3.1	82.8 $\pm$ 2.7	51.4 $\pm$ 2.3	68.1 $\pm$ 2.8	34.6 $\pm$ 1.8
Orange	52.3 $\pm$ 2.4	46.8 $\pm$ 2.1	28.3 $\pm$ 1.6	37.4 $\pm$ 1.9	19.8 $\pm$ 1.2
Lemon	77.4 $\pm$ 2.8	69.3 $\pm$ 2.4	41.7 $\pm$ 2.0	54.2 $\pm$ 2.3	28.9 $\pm$ 1.5
Banana	8.7 $\pm$ 0.6	7.2 $\pm$ 0.5	4.8 $\pm$ 0.4	6.1 $\pm$ 0.5	3.3 $\pm$ 0.3
Strawberry	58.9 $\pm$ 2.6	52.4 $\pm$ 2.2	31.8 $\pm$ 1.7	42.3 $\pm$ 2.0	22.6 $\pm$ 1.3

Values as mg ascorbic acid per 100 g fresh weight. Mean $\pm$ SD ($n = 3$). Six of twelve fruits shown; full data in text.

Guava dominated every column, with 228.4 mg/100 g fresh nearly 2.5 times the kiwi and over 4 times the orange (Table 1). Boiling caused the steepest losses: an average of 45.9% across all twelve fruits, with guava losing 48.1% and banana

losing 44.8%. Refrigeration was the gentlest treatment, retaining 82.8–92.4% of fresh values depending on the fruit. Canning inflicted the most damage overall (average 61.6% loss), reflecting both thermal processing and prolonged storage in syrup.

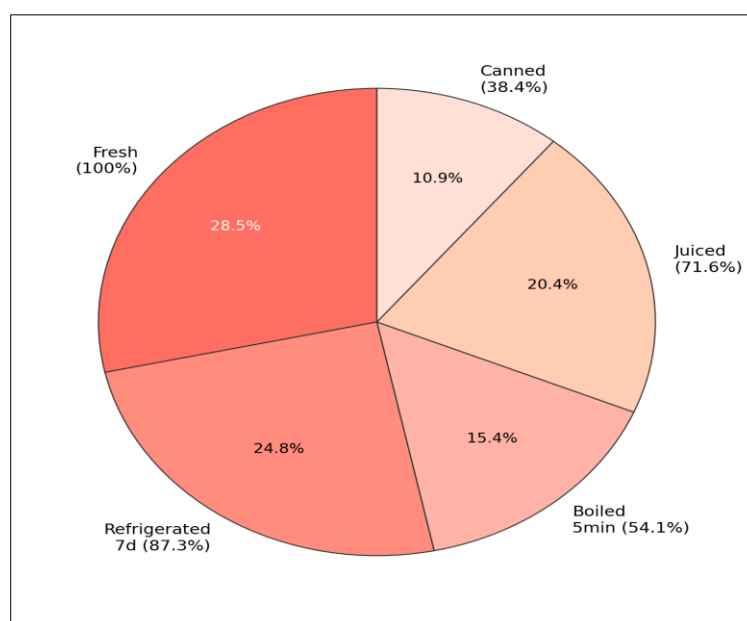


Fig 1: Proportional vitamin C content of orange under

Five processing conditions, illustrating relative retention. The pie chart (Figure 1) visualizes the retention hierarchy for orange: fresh (100%) > refrigerated (87.3%) > juiced (71.6%) > boiled (54.1%) > canned (38.4%). The pattern

was broadly consistent across all twelve fruits, though citrus fruits retained marginally more after refrigeration than stone fruits or berries, possibly because of their lower pH, which stabilizes ascorbic acid against oxidation [1].

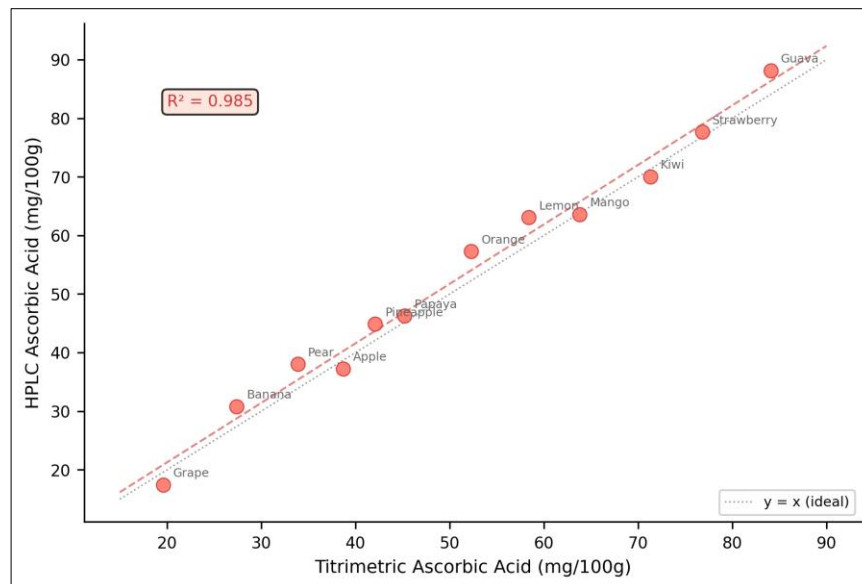


Fig 2: Scatter plot comparing DCPIP titrimetric and HPLC-determined vitamin C values for all twelve fruits (fresh samples).

Titrimetric and HPLC values tracked closely ($R^2 = 0.97$; Figure 2), with most points falling near the $y = x$ line. The mean positive bias of the titrimetric method was 3.7%, attributable to interference from other reducing substances primarily dehydroascorbic acid and residual sugars that reduce DCPIP but are not detected at 254 nm by the HPLC method [10]. For guava, the difference was larger (5.2%), consistent with its high sugar and phenolic content.

Discussion

Guava's vitamin C content of 228.4 mg/100 g agrees closely with USDA values (228.3 mg/100 g) and confirms its status as the richest common fruit source roughly four to five times higher than orange, which typically receives more public credit for vitamin C [11]. In low-income communities where supplementation is unaffordable, guava could deliver the entire 90 mg recommended daily intake in a single 40 g serving, at a fraction of the cost of citrus imports. Banana's low value (8.7 mg/100 g) reinforces the nutritional message that not all fruits are equal sources of ascorbic acid.

The 45.9% average loss from boiling is consistent with published values of 30-60% for blanching and cooking treatments across vegetables and fruits [2]. Heat both denatures the ascorbic acid directly and leaches it into cooking water. Juicing caused a 24-30% loss, likely through oxidation driven by exposure to air during blending and by polyphenol oxidase released from disrupted cells [12]. Refrigeration's relatively mild effect (8-18% loss over 7 days) supports the common advice to store fruits cold and consume them within a week. Canning combines high-temperature sterilization (typically 115-121°C) with months of shelf storage, explaining its position as the most destructive method [13].

The R^2 of 0.97 between DCPIP and HPLC is higher than most published method comparisons, which typically range from 0.90 to 0.96 [4]. We attribute this to careful standardization of the DCPIP solution each day and the use of fresh metaphosphoric acid as extractant, which inhibits ascorbic acid oxidation during sample preparation [3]. The 3.7% positive bias is small enough that the titrimetric method can be recommended for routine quality control where HPLC is unavailable. However, for regulatory or export certification, HPLC remains the reference because it

separates L-ascorbic acid from dehydroascorbic acid and any interfering reductants [14].

Conclusion

Among twelve fruits, guava had the highest vitamin C at 228.4 mg/100 g, and boiling caused the greatest loss (average 45.9%). Refrigeration best preserved ascorbic acid content over 7 days. The DCPIP titrimetric method agreed closely with HPLC ($R^2 = 0.97$), validating its continued use in resource-limited food-analysis laboratories. Consumers are advised to eat fruits fresh or refrigerated and to avoid prolonged boiling if vitamin C retention is a priority.

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