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A REVIEW: ESTIMATION OF VITAMIN-C IN COMMERCIAL AND FRESH FRUIT JUICES BY DIFFERENT ANALYTICAL METHODS

Bumtariya Deepshree Jaykrishna, Rajvi Mahida

ABSTRACT

The study aimed to assess Vitamin C concentration in commercial and fresh fruit juices using various analytical methods. Titration involved the redox reaction of iodine with Vitamin C, while UV-Spectroscopy employed 2,4-DNPH dye coupling. HPLC utilized Acetonitrile-KH2PO4 mobile phase on a Super sphere C18 column. Results indicated higher Vitamin C in lemon and orange, with UV-Spectroscopy showing lemon (3.14 mg/100 ml) > apple (2.78 mg/100 ml) > orange (2.62 mg/100 ml) > grapes (2.2 mg/100 ml) in fresh juices. Titration confirmed orange (41.93 mg/100 ml) > lemon (29.31 mg/100 ml) > apple (26.6 mg/100 ml) > grapes (25.25 mg/100 ml). HPLC revealed lemon (3.316 mg/dl) > orange (3.504 mg/dl) > grapes (2.206 mg/dl) > apple (0.01 mg/dl). Consistent results were observed across methods.

1.INTRODUCTION

Vitamins are essential organic compounds for human metabolism, obtained in small quantities from the diet. Vitamin C, a major water-soluble antioxidant, plays a crucial role in preventing deficiency diseases. It is a 6-carbon organic acid, resembling glucose, with potent reducing agent properties. Citrus fruits are rich sources, and recent research shows higher vitamin C content in human milk than cow's milk.

Absorption and Distribution

Vitamin C is absorbed from the gastrointestinal tract and distributed intra and extra muscularly. Plasma concentration and total body stores depend on daily ascorbic acid intake. The body cannot store more than 2.5g, with higher intake leading to increased urinary excretion.

Metabolism

Ascorbic acid is partially oxidized to active (dehydroascorbic acid) and inactive (oxalic acid) metabolites. Therapeutic uses include preventing deficiency, treating scurvy, aiding in anaemia, acidifying urine in urinary tract infections, lowering blood pressure and cholesterol, and exhibiting beneficial effects in bacterial infections. Adequate intake may also prevent certain cancers.

Sources:

Vitamin C is obtained from fruits and vegetables, crucial for collagen production vital for skin, bone, teeth, and cartilage health. First isolated in 1928, it was proven to prevent scurvy in 1932. Common sources include citrus fruits, tomatoes, broccoli, cauliflower, spinach, and ladyfinger.

Analytical Methods

Various analytical methods such as UV spectrophotometry, chromatography, titrimetry, voltammetry, fluorometry, and potentiometry are used for ascorbic acid determination. UV spectrophotometry is favoured due to its simplicity and Vitamin C's ability to absorb UV rays. It is suitable for vitamin C tablets, fresh or packaged fruit juices, and solid fruits and vegetables.

History of Vitamin C

Discovered in the 1920s by Albert Van Szent Gyorgyi, vitamin C, or ascorbic acid, was identified for its ability to prevent and cure scurvy. Kazimierz Funk had previously included a factor denoted as "C" in his list of vitamins, later identified as ascorbic acid. Despite being crucial for various species, certain animals and humans have lost the ability to produce it during evolution.

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Properties of Vitamin C

Ascorbic acid is a colorless, odorless crystalline substance, soluble in water and alcohol but insoluble in chloroform, ether, and light petroleum. Only the L-isomer has antiscorbutic properties. It is sensitive to oxidation, particularly in the presence of copper and iron. Cooking in copper utensils leads to quick loss, but freezing has no detrimental effect. The oxidative nature makes it a powerful reducing agent, used for various applications like jewerly cleaning in Nigeria.

Biochemical Role of Vitamin C

Vitamin C prevents scurvy by playing a crucial role in collagen formation, maintaining ferrous iron in a reduced form. It is essential for hydroxylation in collagen biosynthesis and influences the activity of other enzymes. While humans and some animals require it as a vitamin, others synthesize it as an intermediate in glucose metabolism.

Excretion

The half-life of ascorbic acid is inversely related to intake, with an average of 16 to 20 days. Kidneys play a major role in excretion and retention. Below 1500 mg, the kidneys efficiently reabsorb, but above 1500 mg, excretion occurs. Plasma levels between 0.8 and 1.4 mg/dl are considered the renal threshold.

2.MATERIALS AND METHODS

Collection and Storage

Fresh fruits (apple, orange, lemon, grapes) were collected from various regions in Nepal. Commercial fruit juices made from these fruits were obtained from a supermarket in Bharatpur. Extraction involved grinding or squeezing, and the obtained juice was used as a sample for analytical procedures.

Different Analytical Methods for Vitamin C Estimation:

1. Phytochemical Screening:
2. TLC Separation:
3. Titrimetric Method:
4. Spectrophotometric Determination:
5. High Performance Liquid Chromatography (HPLC):
6. Iodometry (Iodine Titration):
7. Permanganometric Titration:
8. 2, 6-dichlorophenol Indophenol Method:
9. Sodium Thiosulphate Method:

2.1. Phytochemical Screening

- Conducted on fresh fruit extracts and marketed juices.
- Tested for tri-terpenoids, glycosides, alkaloids, saponins, carbohydrates, flavonoids, tannins, phenols, vitamin C, and protein.
- Standard procedures were followed for screening.

2.2. TLC Separation

- Silica gel GF_{254} plates used with N-butanol: Glacial acetic acid: water (16:4:18 v/v/v) as the solvent.
- Plates dried at 100°C for 5 min, and samples run, followed by spraying with 10% Sulphuric acid in ethanol.
- Rf values calculated.

2.3. Titrimetric Method

- Utilized iodine solution (0.005 mol/l) and starch indicator solution (0.5%).
- Fruit juice titrated with iodine solution using starch indicator until a blue-black color indicated the endpoint.
- Iodine solution standardized using arsenic trioxide, with a factor: 1 ml of 0.05M Iodine = 0.004946 g of As_2O_3.

2.4. UV-Visible Spectrophotometry

- LT-2100 Double beam UV-Visible spectrophotometer with a 10 mm quartz cuvette utilized.
- Spectrophotometric method involved the coupling reaction of 2,4-dinitrophenylhydrazine (DNPH) dye with Vitamin C.
- Samples analyzed at 521 nm, absorbance recorded, and a calibration curve plotted for Vitamin C content using regression analysis.

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2.5. High-Performance Liquid Chromatography (HPLC)
• Method:
• Standard preparation involved dilution of a 1 mg/ml standard solution with the mobile phase.
• Packed juices solutions prepared and analyzed similarly.
• HPLC equipment: Shimadzu LC solution 20A with UV detector at 230 nm.
• Mobile phase: Acetonitrile 40% and KH_2PO_4 60% at pH = 3.
• Column: Supersphere C18 (250 × 4.6mm).
• Mobile phase pumped isocratically at 1.0 ml/min, 25ºC.
• Injection volume: 20µl.
• Ascorbic acid purity: 99.0% from F. Hoffmann–La Roche Ltd.
• Analysis of different branded packed juices purchased locally.

Reagents and Chemicals
• HPLC-grade solvents, spectroscopic-grade chemicals from Merck.
• Pure water produced with a Millipore Milli-Q Plus System.
• Different branded packed juices purchased locally.

2.6. Vitamin C Determination by Iodine Titration (Iodometry) Method
• Reagents:
1. Iodine Solution - Dissolve 5.0 gm Potassium iodide and 0.268 gm Potassium iodate in 200ml distilled water. Add 30 ml of 3M sulphuric acid. Dilute to 500 ml.
2. Vitamin C Std. Solution - Dissolve 0.100 gm Vitamin C in 100ml distilled water.
3. Starch Indicator Solution (1%)
Procedure: 25 ml of Fresh fruit juice or Vitamin C Std. Solution titrated with iodine solution using starch indicator until a persistent blue-violet color is obtained.
• Calculations: Factor: 15.16 ml Iodine solution reacts with 25 mg Ascorbic acid.

2.7. Vitamin C Determination by Iodine Titration (Iodimetry) Method
• Reagents: Iodine solution (0.001N), Vitamin C std. solution (0.5mg/ml), Starch indicator solution (0.5%), sulphuric acid (2M), Potassium iodide (1%).
• Procedure: 2ml of Vitamin C Std. Solution or juice sample titrated with iodine solution using starch indicator until a persistent blue-violet color is obtained.
•Calculations: Factor: 2.7 ml Iodine solution reacts with 1 mg Ascorbic acid.

2.8. Vitamin C Determination by Permanganometric Titration Method
•Reagents: Potassium permanganate solution (0.001N), Vitamin C std. Solution (0.2 mg/ml), Starch indicator solution (0.5%), Sulphuric acid (2M), Potassium iodide (1%).
•Procedure: 4 ml (sample) or 10 ml (Vitamin C Std. Solution) titrated with Potassium permanganate solution using starch indicator until a persistent blue-violet color is obtained.
•Calculations: Factor: 0.1 ml Potassium permanganate solution reacts with 0.8mg Ascorbic acid.

2.9. Vitamin C Determination by 2, 6-dichlorophenol Indophenol Method
•Reagents: 2,6-dichlorophenol indophenol Dye Solution (25mg/100 ml), Vitamin C std. Solution (0.50mg/ml), Metaphosphoric acid (4%).
•Procedure: Titrated Vitamin C Std. Solution or juice sample with 2, 6-dichlorophenol indophenol solution until a persistent rose pink color is obtained.
•Calculations: Factor: 0.1 ml Dye solution reacts with 0.5mg Ascorbic acid.

2.10. Vitamin C Determination by Sodium Thiosulphate Method
•Reagents: Sodium thiosulphate solution(0.1M), Potassium iodate (0.02M), Sulphuric acid (0.5M), Starch indicator solution (1%).

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•Procedure: Sample (Vitamin C powder or juice) titrated with Sodium thiosulphate solution using starch indicator until a persistent blue-violet color is obtained.18
•Calculations: Factor: 15 ml Sodium thiosulphate solution reacts with 100 mg Ascorbic acid.
These methods provide diverse approaches for Vitamin C determination, offering flexibility for different sample types and preferences.

3.RESULTS AND DISCUSSION

3.1 Phytochemical Screening

The phytochemical screening was carried out on various fruits and their results were shownin table 1.

Table 1: Phytochemical screening of fresh fruit Juices

Test			Sample	
Fruits	**Apple**	**Lemon**	**Orange**	**Grapes**
Alkaloids	Yes	Yes	No	Yes
Glycosides	No	Yes	Yes	Yes
Protein	No	No	No	No
Carbohydrates	Yes	No	Yes	Yes
Vitamin C	Yes	Yes	Yes	Yes

3.2 TLC for Qualitative Analysis of Marketed Fruit Juices

Thin Layer Chromatography was carried out as per the procedure discussed above. The TLC plates were dried and the spots were measured after application of visualizing agents.Thus measured values were calculated to obtain Rf values, where all the values showed aneffective separation of Vitamin C as shown in table 2. S_f = distance travelled by solvent, S_p = distance travelled by sample, S_t = distance travelled by standard, R_f = retention factor, [$R_f = S_p/S_f$]

Table 2: TLC for Qualitative Analysis of Marketed Fruit Juices

Fruit	S_f	S_p	S_t	R_f
Orange	10.7	4.3	4.5	0.401
Apple	10.7	5.8	6.0	0.542
Lemon	10.7	5.9	6.0	0.551

3.3 Estimation of Vitamin C Content using UV Spectro-photometry

The Colored complex of the sample was analyzed using double beam spectrophotometer, the absorbance of all standards (converted to colored complex) were taken to construct a calibration curve, as shown in fig.1 The linearity was in compliance with the regression plot in the concentration range of 5–25 µg/ml with a correlation coefficient (R^2) of 0.994. Fig.4 showed the linear graph between concentrations of standard Vitamin C and its absorbance, as per Beer's Lamberts Law.

Determination of Vitamin C Content in Fresh Fruit Juices

Samples of different fruits were prepared and the results of the total content of Ascorbic acid in the investigated samples obtained by the Spectrophotometric methods. The highestcontent of total ascorbic acid obtained by the Spectrophotometric method was found in samples of lemon (3.14 mg/100 ml)>apple (2.78 mg/100 ml)>orange (2.62 mg/100 ml)>grapes (2.2 mg/100 ml) as shown in table 3.

Table 3: UV-Spectrophotometric Method for Estimation of Vitamin C (Fresh FruitJuices)

Sample	Absorbance			Mean (n=3) ±SD	Vit-C content (ug/ml)	Vit-C content (mg/100ml)
	R1	R2	R3			
Apple	0.268	0.267	0.266	0.267±0.001	27.8	2.78
Orange	0.26	0.259	0.258	0.259±0.001	26.2	2.62
Grapes	0.239	0.238	0.237	0.238±0.001	22	2.2
Lemon	0.286	0.285	0.284	0.285±0.001	31.4	3.14

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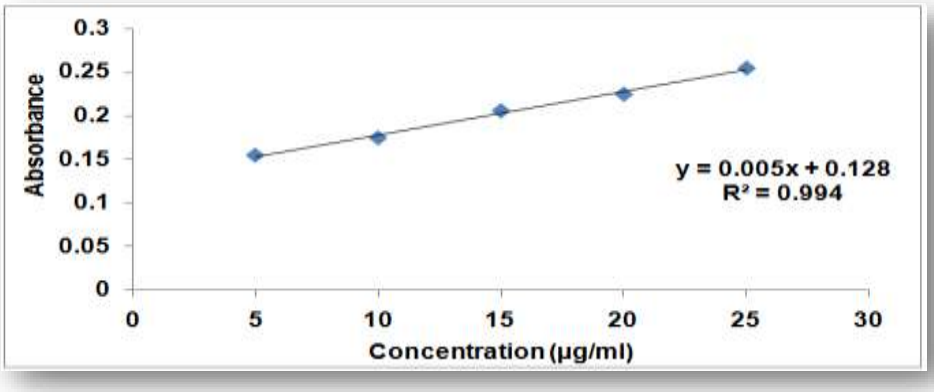
Determination of Vitamin-C Content in Marketed Fruit Juices

Fruit Juices apple, orange, lemon, grapes respectively was brought from market and samples were prepared accordingly. Result of the total content of Ascorbic acid in the investigated samples obtained by Spectrophotometric methods. The highest content of total ascorbic acid obtained by Spectrophotometric method was found in samples of marketed:lemon (1.32 mg/100 ml)>orange (1.24 mg/100 ml)>grapes (1.18 mg/100 ml)>apple (1.14mg/100 ml) as shown in table 4.

Table 4: UV-Spectrophotometric Method for Estimation of Vitamin-C(Marketed Fruit Juices)

Sample	Absorbance			Mean (n=3) ±SD	Vit-C content(ug/ml)	Vit-C content (mg/100ml)
	R1	R2	R3			
Apple	0.186	0.185	0.185	0.185±0.001	11.4	1.14
Orange	0.19	0.191	0.19	0.19±0.001	12.4	1.24
Grapes	0.187	0.187	0.187	0.187±0.001	11.8	1.18
Lemon	0.196	0.196	0.194	0.194±0.001	13.2	1.32

Fig:1 Calibration Curve



3.4 Estimation of Vitamin C Content by Titrimetric Analysis

This method determines the vitamin C concentration in a solution by redox titration usingiodine. As the iodine is added during the titration, the ascorbic acid is oxidized to dehydroascorbic acid, while the iodine is reduced to iodide.

Ascorbic acid+I_2 $\rightarrow$ 2 I^- +dehydroascorbic acid.

Due to this reaction, the iodine formed is immediately reduced to iodide as long as there isany ascorbic acid present. Once all the ascorbic acid has been oxidized, the excess iodine is free to react with the starch indicator, forming the blue-black starch-iodine complex. Thiswas the endpoint of the titration.

Determination of Vitamin-C Content in Fresh Fruit Juices

Samples of different fruits were prepared according to previously written procedure. Results of the total content of Ascorbic acid in the investigated samples were calculated after the completion of titration. The highest content of total ascorbic acid obtained by titration method was found in samples of fresh: orange (41.93 mg/100 ml)>lemon (29.31 mg/100 ml))>apple (26.6 mg/100 ml)>grapes (25.25 mg/100 ml) as shown in table 5.

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Table 5: Vitamin C Content in Various Fresh Fruits

Fresh fruits	Volume of Iodine solution consume(ml)			Mean (n=3)±SD	Mass Of vitamin C(ug/ml)	Mass of vitamin C (mg/100ml)
	S1	S2	S3			
Apple	5.9	5.8	5.9	5.9±0.0577	0.0053	26.60
Orange	9.4	9.3	9.3	9.3±0.0577	0.00838	41.93
Grapes	5.6	5.6	5.6	5.6±0.00	0.0050	25.25
Lemon	6.6	6.5	6.5	6.5±0.0577	0.0058	29.31

Determination of Vitamin-C Content in Marketed Fruit Juices

Fruit Juices with respective apple, orange, lemon, grapes flavoured were brought from market and Samples were prepared according to a previously written procedure on the duedate. Result of the total content of Ascorbic acid in the investigated samples obtained by titrimetric methods. The highest content of total ascorbic acid obtained by titration was found in samples of marketed: orange (46.44 mg/100 ml)>grapes (29.76 mg/100ml)>lemon (24.8 mg/100 ml)>apple (24.35 mg/100 ml), as shown in table 6.

Table 6: Vitamin C Content in Various Marketed Fruits Juices

Sample	Volume of titrant consumed (ml)			Mean (n=3) ±SD	Mass of vitamin C (gm/20ml)	Mass of vitamin C (mg/100ml)
	S1	S2	S3			
Real apple juice	5.3	5.5	5.5	5.4±0.01154	0.00487	24.35
Real Orange Juice	10.2	10.3	10.3	10.3±0.0577	0.00929	46.44
Real Grapes Juice	6.6	6.6	6.6	6.6±0.00	0.00595	29.76
7up nimbooz Juice	3.6	3.5	3.5	3.5±0.0577	0.00496	24.8

3.5 Result of HPLC

Table 7: Vitamin C HPLC Data in Various Fruit.

Sr. No.	Fruit Juices	Conc. (mg/dl) %
1	Lemon	3.502
2	Apple	0.01
3	Orange	3.316
4	Grape	2.025

- Simple isolation technique was followed to collect the juice from fresh fruits using slicing and squeezing technique followed by filtration. Due to a variety of fruits and their content of liquid part, a variation on their yield was observed. As per the data we obtained, there was almost equal yield in fruit juices among apple and grapes with content slight above 50 ml per 100 gm of fruit. However, orange and lemon possess 1/3rd yield of fruit mass which was found to be roughly 27 and 30 ml per 100 gm of fruits,
- Various methods reveal that the fresh fruits juices contained a high amount of Vitamin C than the marketed sample. The absorbance was measured Spectrophotometrically at 521 nm. The Titrimetric method was carried out by an Iodimetric titration. There were varying phytoconstituents among apple, orange, lemon, grapes and their juice content were also different and thin layer chromatography showed their effective separation and HPLC also shows higher concentration in fresh fruit juices.

4. CONCLUSION

In the present study, we found that biologically active phytochemicals were present in every fruit. Various methods for estimation of vitamin C in fruits was a simple and reliable method. Comparison of results obtained by various method was in a good agreement with results obtained by others methods. Though titration method is simple, UV-spectroscopy is less time consuming and easy to interpret as endpoint determination is quite challenging part in iodometric titration and HPLC is also simple method. Marketed fruit juices also contain Vitamin C in considerable amount along with the fresh fruits juices, but degradation on storage was the main point to be noted. This also provides immense guidance to the suppliers and consumers to apply the best storage to the fruits and their juices before consumption and achieve maximum benefit. The highest content of Vitamin C was found in the fresh fruit juices than marketed preparations.

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