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Original Article

# A COMPARATIVE ANALYSIS OF VITAMIN-C CONCENTRATION IN COMMERCIAL FRUIT JUICES AND FRESH FRUITS OF NEPAL WITH EFFECT OF TEMPERATURE

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**ABSTRACT**

**Objective:** It was aimed to determine the best storage temperature for fruits to achieve the highest quality of vitamin-C and to compare vitamin C concentration between commercial fruits juices and fresh fruit juices in by using Spectrophotometry and Titrimetric method

**Methods:** Titration involved the redox reaction between iodine and vitamin C. As the iodine was added during the titration, the ascorbic acid was oxidized to dehydroascorbic acid, while the iodine was reduced to iodide ions. The Spectrophotometric method involved the coupling reaction of 2,4 dinitrophenylhydrazine (DNPH) dye with Vitamin C. The samples were analyzed using UV-Vis-Spectrophotometer at 521 nm.

**Results:** Titrimetric and spectroscopic methods were performed for fresh and marketed fruits comprising of apple, grapes, lemon, orange. The maximum amount was found in lemon and orange whereas apple and grapes contained lesser amount of Vitamin C. The stability of marketed fruit juices made up of apple, grapes, lemon, orange were analyzed by storing them on the freeze at 0 °C and 10 °C and on the hot air oven at 20 °C, 30 °C, 40 °C, 50 °C for 72 h and estimated by both Titrimetric and Spectroscopy method. UV-Spectroscopy method showed that, at freezing condition up to 10 °C temperature, degradation was too low but when the temperature reached 50 °C the extent of degradation was more, showing 24.56% apple juice, 10.89% orange juice,12.70% grapes juice and 50% orange juice were degraded in 100 ml sample. Similar results were observed by analysing the samples with titration technique.

**Conclusion:** A new analytical method was developed to address the content of vitamin C in fruits consumed in the local market of Nepal along with the best possible storage of fruit juice to yield the maximum amount of nutrients.

**Keywords**: Vitamin C, Temperature, UV-Spectroscopy, Titration, Phytochemical, TLC


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## INTRODUCTION

Vitamin C, commonly famous as ascorbic acid is an important food constituent for human health due to its antioxidant activity [1]. Human and animals obtain vitamin C from a wide range of fruits, fruits juices, vegetables. Vitamin C is plentiful in fresh fruit and fruit juices, in particular; citrus fruit, and vegetables. A lack of vitamin C in the diet causes the deficiency disease scurvy [2].

Nowadays, a wide range of ready-to-drink fruit juices prepared from dilution of fresh fruit nectars on the addition of sugars, flavours, preservatives are present in the market. Present-day, different techniques of analysis are applied to estimate the vitamin C content in both the fresh fruit and fruit-based beverages commercially. Ultraviolet (UV) spectroscopy [3-5], High-performance liquid chromatography [6-10], capillary zone electrophoresis [11], voltammetry [12], etc. were proposed to determine vitamin C in the juices and related products. Vitamin C is unstable because of its strong reducing agent property; therefore, it can be deactivated in general oxidizing agents such as atmosphere with oxygen, cooking, high temperature, leaving it uncovered exposed to air and so on [5].

The present study is to evaluate, compare and co-relate the vitamin C concentration between commercial fruit juices and fresh fruit juices along with the determination of best possible storage temperature to retain the marketed juices by allowing them to degrade at varying temperatures.

In this study, vitamin C content in fresh and packaged fruits at different temperatures and the effect of storage and processing of vitamin C in different fruits (lemon, apple, orange, grapes) had been investigated. Recognizing the ongoing consumption of fruits and marketed fruit juices with vitamin C as antioxidants as well as other therapeutic activities, labeled content of vitamin C in commercial fruits juices might not be achieved by consumers because of inappropriate storage conditions.

Commercial fruits juices contain added taste, sugar, nutrition, enzymes, phytochemicals which might get destroyed. Also, Vitamin C can be easily lost by boiling due to its water-solubility and high temperature [7]. This study was carried out to compare and contrast vitamin C concentration between fresh fruits of lemon, apple, orange, grapes of Nepal and the fruits were selected based on the vitamin c content. This technique will be the first in my place to highlight the importance of fruits as per their constituents.

## MATERIALS AND METHODS

### Materials

2, 4 dinitrophenylhydrazine (DNPH) was purchased from Nike chemical, India. Starch, sulphuric acid and ethanol were purchased from Fisher Scientific, Mumbai, India. Potassium iodide was purchased from Emplura, India. Arsenic trioxide was purchased from Lobal Chemie, Mumbai. Ascorbic acid was purchased from Emparta, Merck life science pvt. Ltd. Mumbai. Silica gel GF254 was purchased from Sisco Research laboratory. India. Marketed fruit juices were purchased from Local Bhat-Bhateni Super Market supermarket, Bharatpur, Chitwan.

### Collection and storage

### Fresh fruits

Fresh fruits like apple, orange and grapes were collected from their respective geographical places of Nepal. Apple was collected from the upper Mustang, Nepal. [2,800 to 3,900 m above sea level.], Orange was collected from Salyan, Nepal. [1000m above sea level.], Grapes and lemon were collected from Bharatpur, Nepal. [210m above sea level].

The fruits were stored at room temperature. After extraction and purification, they were stored under varying temperature conditions.

**Commercial fruit juices**

All the commercial fruit juices made up of respective fruits were used for further analysis are collected from Bhatbhateni Supermarket, Bharatpur. These are stored at room temperature before and after analysis and preserved under specified room temperatures as like fresh fruits storage conditions for further analytical procedures.

**Extraction and purification**

A Certain weight of fruit was weighed, sliced, cut down in pieces, grinded or squeezed. This obtained juice was filtrated using filter paper and furthermore used as a sample for analytical procedures.

**Qualitative analysis**

**Phytochemical screening**

Various phytochemical tests were performed for the fresh fruit extracts and marketed juices. The samples were tested for the presence of active principles such as tri-terpenoids, glycosides, alkaloids, saponins, carbohydrates, flavonoids, tannins, phenols, vitamin C and protein. The phytochemical screening was performed by following standard procedures [13, 14].

**TLC separation**

TLC Plates were prepared using silica gel GF254 as stationary phase and N-butanol: Glacial acetic acid: water (16:4:18 v/v/v) was employed as the solvent system. Thus prepared TLC plates were dried at 100 °C for 5 min and then sample and standard ascorbic acid solution were inserted and set to run in the chamber. After the movement of solvent front higher than 2/3$^{rd}$ part, it was sprayed with 10% Sulphuric acid in ethanol. Furthermore, $R_f$ values were calculated [15, 16].

**Quantitative analysis**

**Titrimetric method**

In this experiment, the titration method was used to determine the concentration of Vitamin C from freshly purified fresh juice sample. A titrant of known concentration was used to react with a solution of an analyte to unknown using a calibrated burette. It was possible to determine the exact amount of titrant that has been consumed when the endpoint was reached. The endpoint was the point at which the titration was completed as determined by the color change of indicator [5, 17].

**Preparation of reagents and estimation of Ascorbic acid**

**Iodine solution (0.005 mol/l):** 2 g of potassium iodide was dissolved with water in a 100 ml beaker; 1.3 g of iodine was added in it and dissolved by vigorous shaking. Iodine solution was transferred to a 1 Liter volumetric flask, making sure to rinse all traces of solution into the volumetric flask using distilled water and volume was made to 1 Liter mark with distilled water.

**Starch indicator solution (0.5%):** 0.25 g of starch was weighed and 50 ml of nearly boiling water was added in a beaker to which is dissolved by stirring and cooled before using.

**Assay for vitamin C estimation;** 20 ml of isolated fruit juice sample was taken and 150 ml of water was added to the sample in a conical flask. The solution is titrated with 0.005 mol/l Iodine solution using the starch solution as an indicator till blue-black color is reached.

**Standardization of iodine solution**: 0.15 gm of arsenic trioxide previously dried at 105 °C for 1 hour was dissolved in 20 ml of 1M NaOH, diluted with 40 ml of water, 0.1 ml of methyl orange solution was added and the add dilute hydrochloric acid was added drop wisely until yellow color changed to pink. To the solution, 2g of sodium carbonate and 50 ml water along with a 3 ml starch solution was added. Titration was continued with Iodine solution until a permanent blue color is produced.

1 ml of 0.05M Iodine is equivalent to 0.004946g of $As_2O_3$.

**Spectrophotometric determination**

LT-2100 Double beam UV-visual spectrophotometer with 10 mm quartz cuvette was used to record the absorbance. The Spectrophotometric method involved the coupling reaction of 2,4 dinitrophenylhydrazine (DNPH) dye with Vitamin C followed by Spectrophotometric determination. The samples were analyzed using UV-Vis-Spectrophotometer at 521 nm and absorbance was taken and the calibration curve was plotted, followed by calculation of Vitamin C content using Regression analysis [3].

**RESULTS AND DISCUSSION**

**Estimation of ascorbic acid content using UV-spectrophotometry**

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. 1 showed the linear graph between concentrations of standard Ascorbic acid and its absorbance, as per Beer's Lamberts Law.

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

**TLC for qualitative analysis of marketed fruit juices**

Thin Layer Chromatography was carried out as per the procedure discussed above [13, 14]. The TLC plates were dried and the spots were measured after application of visualizing agents. Thus measured values were calculated to obtain $R_f$ values, where all the values showed an effective separation of Vitamin C as shown in table 2.

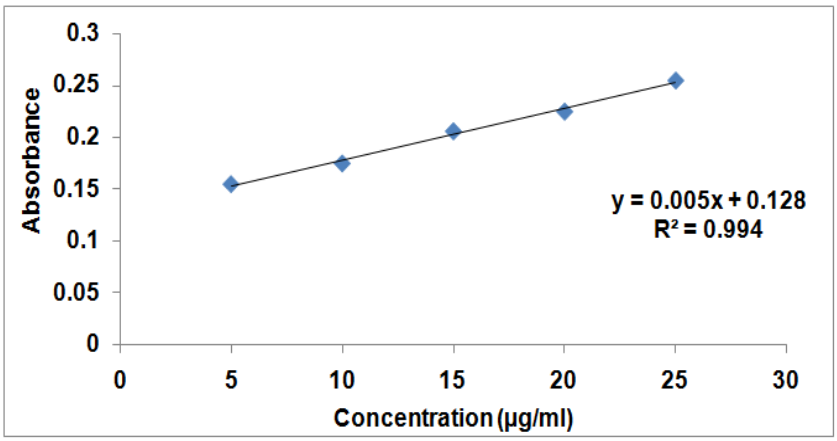


**Fig. 1: Calibration curve of ascorbic acid**

**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 |
| Tannins and phenols | Yes | Yes | Yes | Yes |
| Saponins | No | No | No | No |
| Flavonoids | Yes | Yes | Yes | No |
| Carbohydrates | Yes | No | Yes | Yes |
| Tri-terpenoids | No | Yes | No | Yes |
| Vitamin-C | Yes | Yes | Yes | Yes |

Yes indicates present, No indicates absent

**Table 2: TLC for qualitative analysis of marketed fruit juices**

| Fruit | Sf | Sp | St | Rf |
|---|---|---|---|---|
| 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 |
| Grapes | 11.0 | 5.6 | 6.0 | 0.509 |

Sf=distance traveled by solvent, Sp=distance traveled by sample, St=distance traveled by standard, $R_f$= retention factor, [Rf = Sp/Sf]

**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 highest content 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 fruit juices)**

| Sample | Absorbance | | | Mean (n=3)±SD [a] | Vit-C Content (µg/ml) | Vitamin-C Content (mg/100 ml) |
|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | | | |
| 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 |

[a]Mean for three independent analyses, Vit-C= Vitamin C $R_1$, $R_2$, $R_3$= three respective trials, S. D = standard deviation

**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(1.14 mg/100 ml) as shown in table 4.

**Table 4: UV-Spectrophotometric method for estimation of vitamin-C (marketed fruit juices)**

| Sample | Absorbance | | | mean±SD[a] | Vitamin C content (µg/ml) | Vitamin C content (mg/100 ml) |
|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | | | |
| Real Apple | 0.186 | 0.185 | 0.185 | 0.185±0.0005 | 11.4 | 1.14 |
| Real Orange | 0.19 | 0.191 | 0.19 | 0.19±0.0005 | 12.4 | 1.24 |
| Real Grapes | 0.187 | 0.187 | 0.187 | 0.187±0.0001 | 11.8 | 1.18 |
| 7UP Nimbooz | 0.196 | 0.196 | 0.194 | 0.194±0.001 | 13.2 | 1.32 |

[a]Mean for three independent analyses, $R_1$, $R_2$, $R_3$= three respective trials, SD= standard deviation, 7up nimbooz= marketed lemon juice

**Determination of Vitamin C content under the varying temperature condition**

The marketed fruit juices of apple, grapes, lemon, orange were stored on the freeze at 0 °C and 10 °C and on the hot air oven at 20 °C, 30 °C, 40 °C, 50 °C for 72 h, as shown in table 5. After completion of the storage, samples were prepared and analyzed for the determination of ascorbic acid content using double beam spectrophotometer. The solution was diluted to obtain the concentration of 15µg/ml solution, similar to fresh fruits.

**Table 5: UV-Spectrophotometric method for estimation of vitamin-C under varying temperature conditions (Marketed fruit juices)**

| Sample | Temp | Absorbance | | | mean±SD[a] | Vit-C | Vit C | |
|---|---|---|---|---|---|---|---|---|
| | | Trial 1 | Trial 2 | Trial3 | | content µg/ml | content mg/100 ml | Degradation in % |
| Real apple juice | 0 °C | 0.189 | 0.189 | 0.188 | 0.189±0.0005 | 12.2 | 1.22 | |
| | 10 °C | 0.192 | 0.191 | 0.19 | 0.191±0.001 | 12.6 | 1.26 | 95.08 % at 50 °C |
| | 20 °C | 0.182 | 0.180 | 0.179 | 0.180±0.0005 | 10.4 | 1.04 | |
| | 30 °C | 0.169 | 0.169 | 0.168 | 0.169±0.0005 | 8.2 | 0.82 | |
| | 40 °C | 0.148 | 0.147 | 0.147 | 0.147±0.0005 | 3.8 | 0.38 | |
| | 50 °C | 0.131 | 0.131 | 0.132 | 0.131±00.005 | 0.6 | 0.06 | |
| Real orange | 0 °C | 0.199 | 0.198 | 0.198 | 0.198±0.0005 | 14 | 1.4 | |
| Juice | 10 °C | 0.201 | 0.201 | 0.202 | 0.201±0.0005 | 14.6 | 1.46 | 64 % at 50 °C |
| | 20 °C | 0.192 | 0.191 | 0.193 | 0.192±0.001 | 12.8 | 1.28 | |
| | 30 °C | 0.181 | 0.181 | 0.181 | 0.181±0.0001 | 10.6 | 1.06 | |
| | 40 °C | 0.161 | 0.161 | 0.162 | 0.161±0.005 | 6.6 | 0.66 | |
| | 50 °C | 0.154 | 0.153 | 0.152 | 0.153±0.001 | 5 | 0.5 | |
| Real grapes juice | 0 °C | 0.182 | 0.181 | 0.181 | 0.181±0.0005 | 10.6 | 1.06 | |
| | 10 °C | 0.16 | 0.161 | 0.161 | 0.161±0.0005 | 6.6 | 0.66 | 83 % at 50 °C |
| | 20 °C | 0.178 | 0.173 | 0.173 | 0.17±0.0028 | 9.2 | 0.92 | |
| | 30 °C | 0.154 | 0.153 | 0.152 | 0.153±0.001 | 5 | 0.5 | |
| | 40 °C | 0.149 | 0.149 | 0.148 | 0.149±0.0005 | 4.2 | 0.42 | |
| | 50 °C | 0.138 | 0.136 | 0.136 | 0.137±0.001 | 1.8 | 0.18 | |
| 7up nimbooz | 0 °C | 0.179 | 0.178 | 0.179 | 0.179±0.0005 | 10.2 | 1.02 | |
| | 10 °C | 0.176 | 0.176 | 0.175 | 0.176±0.0005 | 9.6 | 0.96 | 72.5 % at 50 °C |
| | 20 °C | 0.175 | 0.176 | 0.175 | 0.175±0.0005 | 9.4 | 0.94 | |
| | 30 °C | 0.174 | 0.173 | 0.173 | 0.173±0.0005 | 9 | 0.9 | |
| | 40 °C | 0.154 | 0.153 | 0.153 | 0.153±0.0005 | 5 | 0.5 | |
| | 50 °C | 0.142 | 0.141 | 0.142 | 0.142±0.0005 | 2.8 | 0.28 | |

7up nimbooz= marketed lemon juice, S.D. = standard deviation, [a]Mean for three independent analyses, Temp= Temperature

In almost all fruit juices, it was found the gradual loss of vitamin C content on increasing temperature though there is a minor retaining possibility of Vitamin C. We found there was a huge difference in degradation ratio and pattern as per enhancing heat. At freezing condition up to 10 °C temperature, degradation is too low but when the temperature reaches 50 °C, the extent of degradation increases.

**Estimation of Vitamin C content by titrimetric analysis**

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

Ascorbic acid+$I_2$ → 2 $I^-$+dehydroascorbic acid

Due to this reaction, the iodine formed is immediately reduced to iodide as long as there is any 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. This was 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 6.

**Table 6: Vitamin C content in various fresh fruits**

| Fresh fruits | Volume of Iodine solution consumed (ml) | | | Mean±S.D.[a] | Mass of vitamin C gm/20 ml | Mass of vitamin mg/100 ml |
|---|---|---|---|---|---|---|
| | 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 |

[a]Mean for three independent analyses, $S_1$, $S_2$, $S_3$= three respective trials, SD= standard deviation

**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 due date. 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/100 ml)>lemon (24.8 mg/100 ml)>apple (24.35 mg/100 ml), as shown in table 7.

**Determination of vitamin-C content in marketed fruit juices**

Thus received marketed fruit juices made up of apple, grapes, lemon, orange were stored on the freeze at 0 °C and 10 °C and on the hot air oven at 20 °C, 30 °C, 40 °C, 50 °C for 72 h. After completion of storage, samples were prepared and analyzed for determination of ascorbic acid using titrimetric as like fresh fruit juice. The content of ascorbic acid in each fruit juice sample after storage at varying temperature is shown in table 8.

**Table 7: Vitamin C content in various marketed fruits juices**

| Sample | Volume of titrant consumed | | | | Mass of vitamin C gm/20 ml | Mass of vitamin C mg/100 ml |
|---|---|---|---|---|---|---|
| | $S_1$ | $S_2$ | $S_3$ | mean±S.D. | | |
| Real apple juice | 5.3 | 5.5 | 5.5 | 5.4±0.1154 | 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 | 0.005953 | 29.76 |
| 7up nimbooz | 3.6 | 3.5 | 3.5 | 5.5±0.0577 | 0.004961 | 24.8 |

[a]Mean for three independent analyses, $S_1$, $S_2$, $S_3$= three respective trials, SD= standard deviation

**Table 8: Vitamin-C content under varying temperature conditions in marketed fruit juices (titrimetric analysis)**

| Sample | Temperature | Volume of titrant consumed | | | Mean±SD[a] | Vit-C content µg/ml | Vit C content mg/100 ml | Degradation in % |
|---|---|---|---|---|---|---|---|---|
| | | Trial 1 | Trial 2 | Trial 3 | | | | |
| Real apple juice | 0 °C | 5.3 | 5.3 | 5.4 | 5.3±0.0577 | 0.0047 | 23.90 | |
| | 10 °C | 4.7 | 4.8 | 4.9 | 4.8±0.100 | 0.004 | 21.64 | 24.56% at 50 |
| | 20 °C | 4.8 | 4.7 | 4.7 | 4.7±0.0577 | 0.0042 | 21.19 | °C |
| | 30 °C | 4.6 | 4.6 | 4.6 | 4.6±0.00 | 0.0041 | 20.74 | |
| | 40 °C | 4.2 | 4.3 | 4.4 | 4.3±0.100 | 0.0038 | 19.39 | |
| | 50 °C | 4.0 | 4.0 | 4.1 | 4±0.0577 | 0.0036 | 18.03 | |
| Real orange Juice | 0 °C | 10.1 | 10.2 | 10.1 | 10.1±0.0577 | 0.0091 | 45.54 | |
| | 10 °C | 10.0 | 10.0 | 10.0 | 10.0±0.00 | 0.0090 | 45.09 | 10.89% at 50 |
| | 20 °C | 9.8 | 9.7 | 9.7 | 9.7±0.0577 | 0.0087 | 43.74 | °C |
| | 30 °C | 9.4 | 9.5 | 9.3 | 9.4±0.100 | 0.0084 | 42.38 | |
| | 40 °C | 9.4 | 9.4 | 9.5 | 9.4±0.0577 | 0.0084 | 42.38 | |
| | 50 °C | 9.0 | 9.0 | 9.1 | 9±0.0577 | 0.0081 | 40.58 | |
| Real grapes juice | 0 °C | 6.4 | 6.3 | 6.3 | 6.3±0.0577 | 0.0056 | 28.41 | |
| | 10 °C | 6.3 | 6.3 | 6.2 | 6.3±0.0577 | 0.0056 | 28.41 | 12.70% at 50 |
| | 20 °C | 6.3 | 6.2 | 6.0 | 6.2±0.1527 | 0.0055 | 27.95 | °C |
| | 30 °C | 6.0 | 6.0 | 6.0 | 6±0.00 | 0.0054 | 27.05 | |
| | 40 °C | 6.0 | 6.0 | 6.1 | 6±0.0577 | 0.0054 | 27.05 | |
| | 50 °C | 5.5 | 5.5 | 5.6 | 5.5±0.0577 | 0.0049 | 24.80 | |
| 7up nimbooz | 0 °C | 3.0 | 3.0 | 3.0 | 3±0.00 | 0.0027 | 13.52 | |
| | 10 °C | 2.8 | 2.9 | 2.8 | 2.8±0.0577 | 0.0025 | 12.62 | 50% at 50 °C |
| | 20 °C | 2.8 | 2.6 | 2.6 | 2.6±0.1154 | 0.0023 | 11.72 | |
| | 30 °C | 2.3 | 2.2 | 2.2 | 2.2±0.0577 | 0.0019 | 9.92 | |
| | 40 °C | 2.2 | 2.2 | 2.3 | 2.2±0.0577 | 0.0019 | 9.92 | |
| | 50 °C | 1.5 | 1.5 | 1.6 | 1.5±0.0577 | 0.0013 | 6.76 | |

[a]Mean for three independent analyses, SD= standard deviation, 7up nimbooz= marketed lemon juice

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 1/3[rd]yield of fruit mass which was found to be roughly 27 and 30 ml per 100 gm of fruits, as shown in fig. 2.

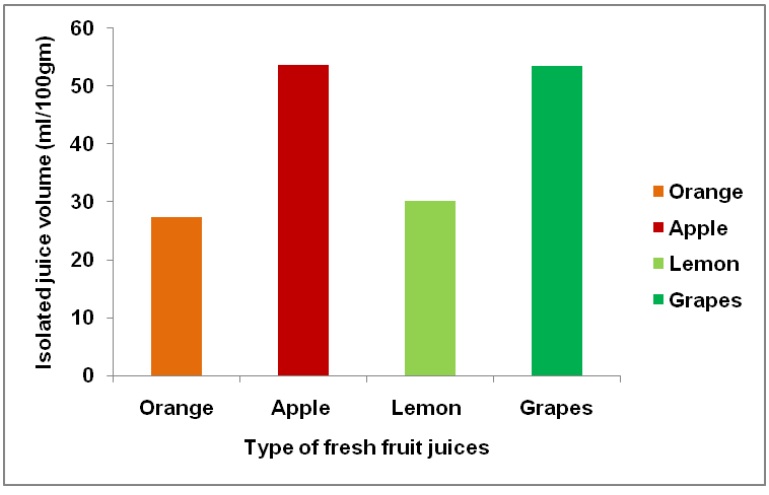


**Fig. 2: Representation of variation of fruit Juices among various fruits**

UV spectroscopy and titration revel that the fresh fruits cultivated in Nepal 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.

In almost all fruit juices, it was found the gradual loss of vitamin C content on increasing temperature though there is not any retaining possibility of Vitamin C. We found there was a huge difference in degradation ratio and pattern as per enhancing heat. At freezing condition up to 10 °C temperature, degradation is too low but when the temperature reaches 20 °C extent of degradation increases and near to 50 °C ascorbic acid is highly degraded.

It is obvious that with increasing temperature, the amount of vitamin C decreases, which is in agreement with previous studies [7]. Several studies show different storage conditions are of value for decreasing the damaging effect on the quality of the product. The various marketed fruits products were exposed to different environmental conditions, especially the fruits sellers who used to sell those fruit products in such a high temperature (about 40-45 °C) there was definitely the loss of vitamins. Hence, this study cautiously deals with temperature as much influential factor for Vitamin C degradation.

## CONCLUSION

In the present study, we found that biologically active phytochemicals were present in every fruit. Spectrophotometric method for estimation of vitamin C in fruits with 2, 4-DNPH was a simple and reliable method. Comparison of results obtained by Spectrophotometric method was in a good agreement with results obtained by titrimetric method and literature values. 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. Marketed fruit juices also contain vitamin C in considerable amount along with the fresh fruits, 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.

## AUTHORS CONTRIBUTIONS

Hemraj Sharma: Designed the study, wrote the first draft, gave the answer of all reviewers comment and approved the final version to be submitted.

Hari Prasad Sapkota: Helped in literature searches and helped in correction and designing of a draft to the manuscript.

Ashish Khanal: Performed all experiments, collected data and submitted as a draft.

Omraj Dhakal: Helped in literature searches, performed all experiments.

Rupa Gurung: Performed all experiments, helped in literature searches

## CONFLICT OF INTERESTS

The authors report no conflicts of interest

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