



Comparative Stability Study of Vitamin C Present in Fresh Lemon Juice and Marketed Juice by Analytical Methods

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Abstract: Determine the stability of vitamin C in commercially available juice and fresh lemon juice, and compare the outcomes of iodometry and colorimetry. Fresh lemon juice (also known as lemon juice with salt or lemon juice with sugar and salt) was manufactured, and commercial juice (Dabur and Freshzo) was bought from the market and kept in glass containers at room temperature and in the refrigerator. Colorimetry and iodometry were used to determine the amount of ascorbic acid contained in each juice. 13 days were dedicated to the investigation in the months of March for reproducibility and January for stability testing. Comparing ascorbic acid degradation at ambient temperature and refrigerated settings, this study finds that the former is less common.

KEYWORDS: LEMON JUICE, ASCORBIC ACID, IODIMETRY, COLORIMETRY, ROOM TEMPERATURE, REFRIGERATOR, STORAGE, STABILITY AND DEGRADATION.

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 2/3rd 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-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 (R²) 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.

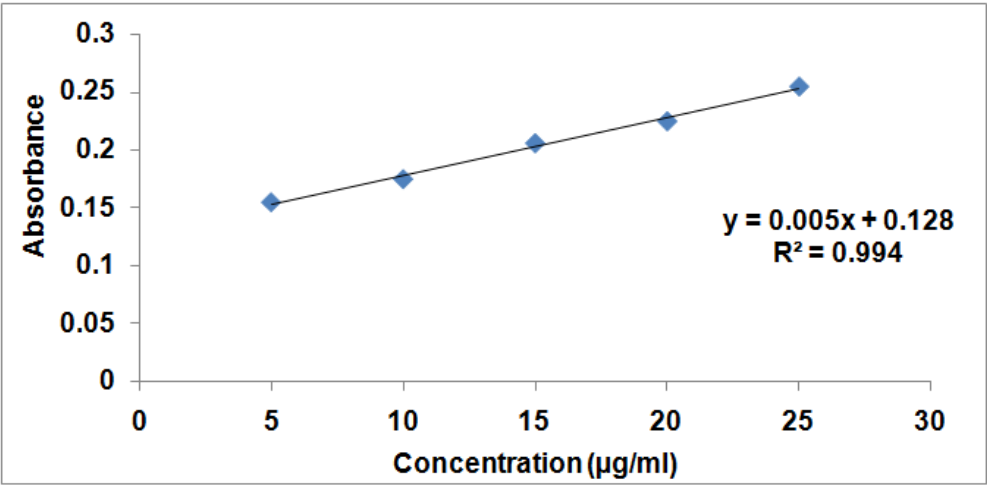


Fig. 1: Calibration curve of ascorbic acid

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, [R_f = 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

Mean for three independent analyses, Vit-C= Vitamin C R₁, R₂, R₃= 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

^aMean for three independent analyses, R₁, R₂, R₃= 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)

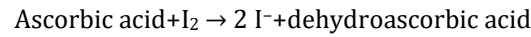
Sample	Temp	Absorbance			mean±SD ^a	Vit-C	Vit C	Degradation in %
		Trial 1	Trial 2	Trial3		content µg/ml	content mg/100 ml	
Real apple juice	0 °C	0.189	0.189	0.188	0.189±0.0005	12.2	1.22	95.08 % at 50 °C
	10 °C	0.192	0.191	0.19	0.191±0.001	12.6	1.26	
	20 °C	0.182	0.180	0.179	0.180±0.0005	10.4	1.04	
	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 Juice	0 °C	0.199	0.198	0.198	0.198±0.0005	14	1.4	64 % at 50 °C
	10 °C	0.201	0.201	0.202	0.201±0.0005	14.6	1.46	
	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	83 % at 50 °C
	10 °C	0.16	0.161	0.161	0.161±0.0005	6.6	0.66	
	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	72.5 % at 50 °C
	10 °C	0.176	0.176	0.175	0.176±0.0005	9.6	0.96	
	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, ^aMean 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.



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

Mean for three independent analyses, S₁, S₂, S₃= 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

^aMean for three independent analyses, S₁, S₂, S₃= 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	24.56% at 50 °C
	10 °C	4.7	4.8	4.9	4.8±0.100	0.004	21.64	
	20 °C	4.8	4.7	4.7	4.7±0.0577	0.0042	21.19	
	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	10.89% at 50 °C
	10 °C	10.0	10.0	10.0	10.0±0.00	0.0090	45.09	
	20 °C	9.8	9.7	9.7	9.7±0.0577	0.0087	43.74	
	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	12.70% at 50 °C
	10 °C	6.3	6.3	6.2	6.3±0.0577	0.0056	28.41	
	20 °C	6.3	6.2	6.0	6.2±0.1527	0.0055	27.95	
	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	50% at 50 °C
	10 °C	2.8	2.9	2.8	2.8±0.0577	0.0025	12.62	
	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	

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

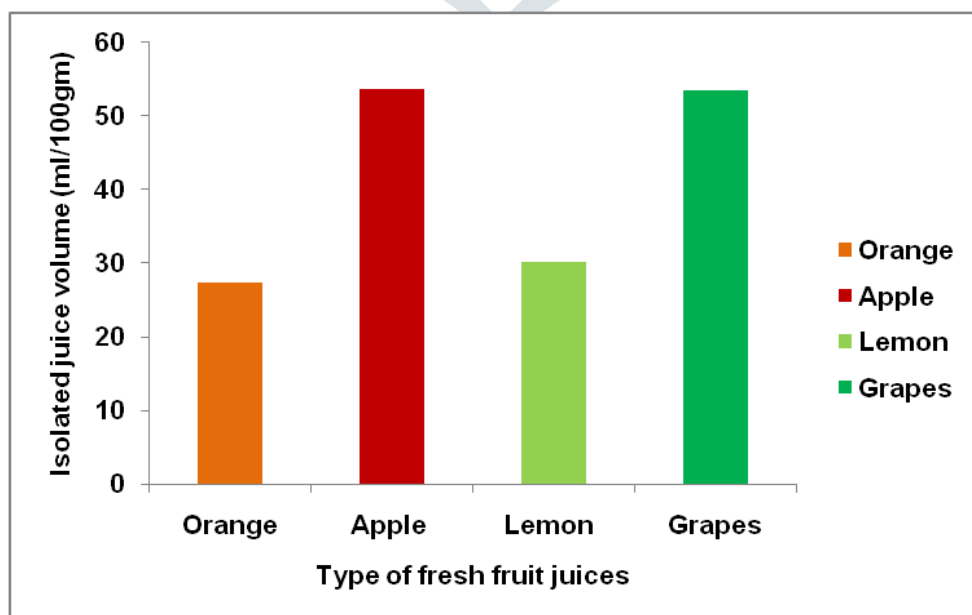


Fig. 2: Representation of variation of fruit Juices among various fruits

UV spectroscopy and titration reveal 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 reviewer's comments, 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, and performed all experiments.

Rupa Gurung: Performed all experiments, helped in literature searches.

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