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Research Article

ACTIVE CONTENT VARIATION IN CITRUS LEMON: AGE, TEMPERATURE, PH AND AIR

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ABSTRACT

Vitamin C is an antioxidant and free radical scavenger for human body. Consumption of Vita C as a nutrient in diet is recommended. Thermal process, environmental factors and pH can cause a negative impact on vitamin content in multivitamin formulation and juices due to physical and chemical instability. Vita C content varies with the age of lemon fruit from unripe to stale. The present paper aims towards content variability and stability studies under various storage conditions like temperature air and pH. Classical methodology like Iodatometric titration is an easy way to trace out the vitamin content in any source using potassium iodate with starch as external indicator. Fresh ripened lemon juice contained 36.5 mg/100ml Vita C, whereas unripened contains 74.6 mg/100ml. Thermal shock of higher degree can decrease the content but for preservation lower temperature was found less deteriorative and deleterious. Percent loss of vitamin were found higher, 56.52 at 80°C and 39.12, 34.8 and 26.3 at 60°C, 40°C and room temperature respectively. Lower temperature shows good retention of vitamin content in the juice so as the percent loss were found 21.9, 13.01 and 8.68 at 12°C , 0°C and -12°C respectively. Air treatments and pH attacks were found harmful for Vita C storage. Storage of it in open bottle in environment the amount was decrease up to with time of min. manipulation of ph using acids and bases also found to decrease the content.

KEYWORDS*:* Vitamin C Iodatometric, Age, Thermal Shocks, Storage, Environmental and Deteriorative.

INTRODUCTION

Discovery of Vitamin C [Vita C] in 1932 comes with a revolutionary mark for sailor's disease called scurvy. Literature tells about the instability of vitamins with heat, pH and long term storage at inappropriate conditions. Vita C is one of very thermolabile and more unstable vitamin among all. It is a white crystalline water soluble vitamin, chemically lactones derivative having enediol as functional group [1] It controls infections and the body's responses to stress. It is a very good easily available nutritional antioxidant agent that diminishes free radicals. Titration or volumetric analyses are common and classical laboratory methods for quantitative estimation. These are very easy and economic less tedious methods for quantitation. A titrant is reagent of known concentration is used to react with a solution of the analyte [Titrand] of unknown concentration. Using a calibrated burette, it is possible to determine the exact amount of titrant that has been consumed to achieve a clear and accurate endpoint. The endpoint or the "equivalence point" tells about the completion of titration by visual color change of the indicator [7] Nutritional Quality of foods during storage is very much crucial aspect to be discussed. Loss of some important and vital vitamins during processing and storage in marketed multivitamin juices and formulation can be determined by using any quantitation method [11] Citrus juices containing ascorbic acid [Vita C] is one of the most sensitive and instable if condition specified are not fulfilled. In this experiment, we use classical redox or iodatometric

titration method to determine the concentration of Vita C. This research paper uses a classical and easy method for quantitation of Vita C in various conditions. Degradation of Ascorbic acid [AA] proceeds both aerobically and anaerobically depend upon many factors such as oxygen, heat, light, storage temperature and storage time. Anaerobic degradation of AA mainly appears during storage which is especially observed in thermally preserved citrus juices. It was reported that several decomposition ends in production of brown pigments which are mainly hydroxyl methyl furfural [2][3]

PRINCIPLE AND MECHANISM

Vitamin C always acts with iodine but there should be acidic [Sulphuric] environment. It should not be titrated directly with iodine due to the low solubility of iodine in aqueous media. Iodatometric is better than iodimetry because there is no need for standardization like in iodimetry [7] The iodine is generated indirectly by below written reaction:-

I_2 production: Potassium iodide [KI], Sulphuric acid [$H2SO_4$] and Potassium Iodate [KIO3]

$KIO_3 + 5KI + 6\ H2SO_4 \rightarrow 3I_2 + 3H_2O$

Oxidation reaction: Produced liberated iodine and vitamin C gives oxidized ascorbic acid

$I_2 + C_6H_8O_6$ [Vitamin C] $\rightarrow C_6H_6O_6$ [Oxidized Vitamin C] + 2HI

Color producing reaction: It is endpoint detection reaction of starch and unused iodides

$C_6H_8O_6 + I_2 \rightarrow$ No more reaction, due to exhaustion of vitamin in reaction vessel then

I_2 [unused] + KI $\rightarrow I^{3-}$ or I^{4--}

I^{3-} or I^{4--} + Starch $\rightarrow$ iodide–starch complex $\rightarrow$ deep blue color [endpoint].

MATERIAL AND METHODS

Materials

Glasswares And Equipments: Classical method for estimation of vitamins needs burette, conical, pipettes, juice pressing machine, filter papers, buchner funnel, glass stopper containers, hot plate, hot air oven, digital freezer, pH meter & thermometer Weighing bottle & digital balance [mg].

Chemicals: Standard Vitamin C [Ascorbic Acid] and other chemicals like Iodine crystals, Potassium Iodate, Concentrated Sulphuric acid Hydrochloric acid, Sodium hydroxide, and Starch powder are kindly provided in house. Distilled water was obtained from in house laboratory. Chemicals and solvents used were of A.R. grade.

Methods

Preparation Of Indicator Solutions

Add 1.0 g of solid starch powder to 50 ml of boiling distilled water. Mix well and add the remaining hot water up to the mark of volumetric. Allow to cool before use. It should be freshly prepared if exhausted. [7][8]

Preparation Of Titrant Solutions

Add solid Potassium iodide [10g] and Potassium Iodate [1.07g] in 100 ml water. Add 3M of Sulphuric acid [50 ml] and stir well. Make the volume up to 1000 ml to produce 0.005M solution. Store in well closed volumetric flask in a cupboard box[7][8]

Preparation Of Standard Vitamin C Solution

Dissolve standard Vitamin [500 mg] in 100ml water then make the volume up to 500ml with distilled water to produce 1 mg/ml in well closed and glass stopper volumetric flask in cupboard box[8]

Preparation of pH modulating agents

Take 8.5ml of concentrated Hydrochloric acid and dissolve this in 100 ml water in a volumetric flask to get 0.1 M HCl whereas for 0.1M Sodium hydroxide, take 4 gm of solid pellets in 100 ml volumetric flask. Make the volume up to mark with distill water to produce 0.1 M NaOH [9]

Titration of standard Vitamin C

Titration of standard Vitamin C solution [10ml] in conical flask against iodine solution with starch solution as external indicator. Indicator should be added nearby endpoint for proper endpoint of blue color. Repeat the titration at least twice.

Determination of the Vitamin C in a fresh lemon juice

Cut the lemons of different ages [Unripened, Semiripened, Ripened and Stale] in two halves. Press the both half in manual press and squeeze out the juice. Filter and remove the seeds and fleshes with aid of muslin cheese cloth [at least 10ml lemon juice should be collected].Pipette out required amount of each type of the juice into a conical flask. Titrate it individually with iodine solution. Triplicate the test readings and average the results. Calculate the Vitamin C content in mg per 100 ml with below written formula.

$$\text{Vitamin C [mg/ml]} = \frac{\text{Titrant used for 10 ml sample}}{\text{Titrant used for standard 10 ml Vita C}} \quad (1)$$

$$\text{Vitamin C [mg/100 ml]} = \text{Amount in mg}/_{\text{ml}} \times 100 \quad (2)$$

Factor = 100 [250 mg standard in 250 ml solvent so stock is 1 mg/ml but for mg/100ml, multiplied by 100]

Quantitation Of Vitamin C And Thermal Treatments

Cut the ripened lemons in to two halves and squeeze out the juice. Filter it to remove undesired material. Pipette out the juice [10 ml] from stock sample and titrate it initially [Day 1], labeled it zero reading. The juice is then titrated at specified interval after storing it in various temperature [± 3°C] conditions like RT, 40, 60, 80, 0, 12 and -12°C. The temperature variation can be done with the help of ice bath and organic solvents as shown in Table 1 Calculate the percent change in Vitamin C content by above given formula. Time interval is large for normal or freezing temperatures but very close and less for higher degrading temperature, normally assuming thermal degradation so that time interval has designed as such. Data are depicted in figures below [Fig 1-7].

Table 1: *Storage and thermal treatment conditions*

S. no	Storage thermal condition*	Measures	Study time period [hours]
1	-12°C	Ice + Ethanol +Ethylene glycol	96 [Four days]
2	0°C	Crushed Ice	96 [Four days]
3	12°C	Ice + Xylene	96 [Four days]
4	Room temperature [27±2°C]	Lab itself	96 [Four days]
5	40°C	Dry oven*	4 [Single day]
6	60°C	Dry oven	4 [Single day
7	80°C	Dry oven	4 [Single day

*20 minutes is storage time at each condition in dry place

Figure 1: *Stability curve at room temperature*

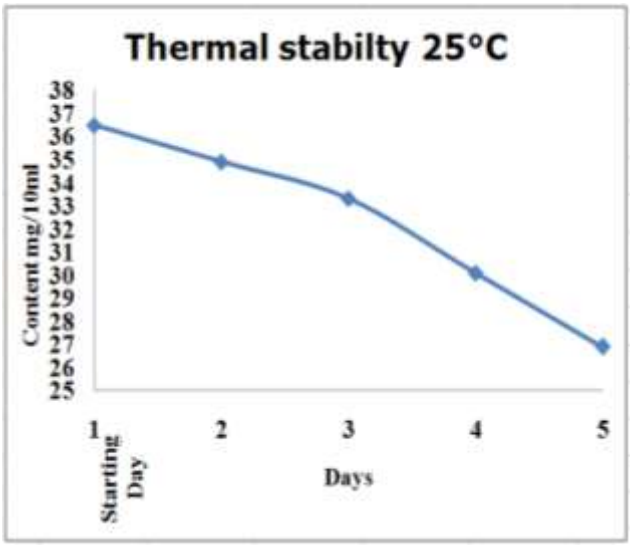


Figure 2:*Stability curve at 40°C*

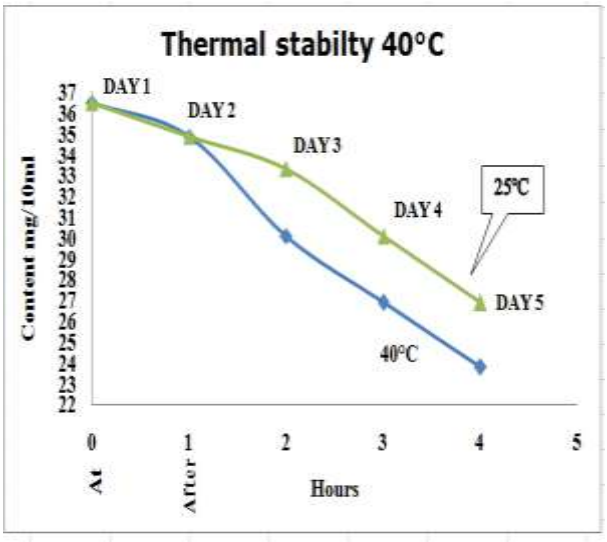


Figure 3: *Stability curve at 60°C*

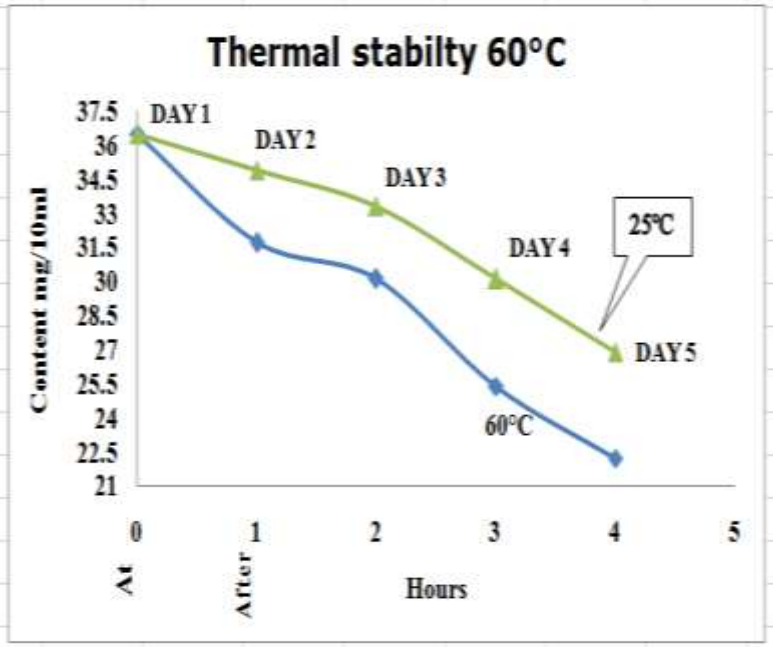


Figure 4: *Stability curve at 80°C*

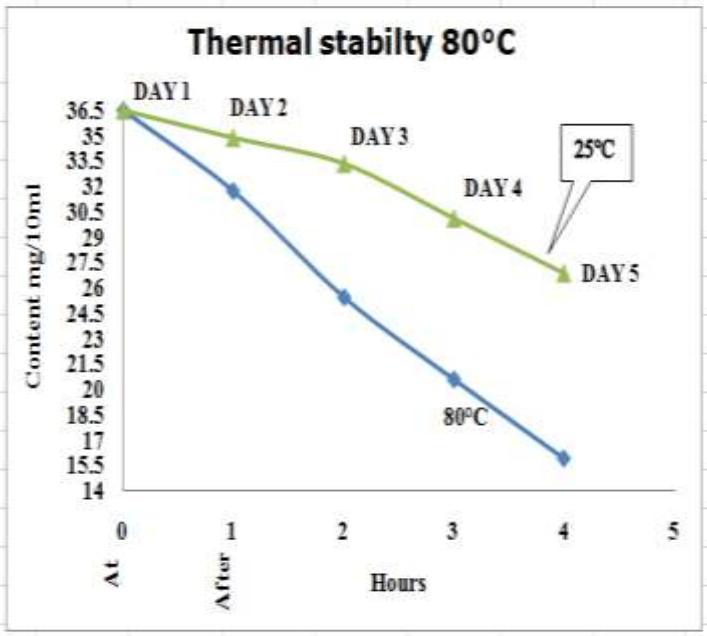


Figure 5: *Stability curve at 0°C*

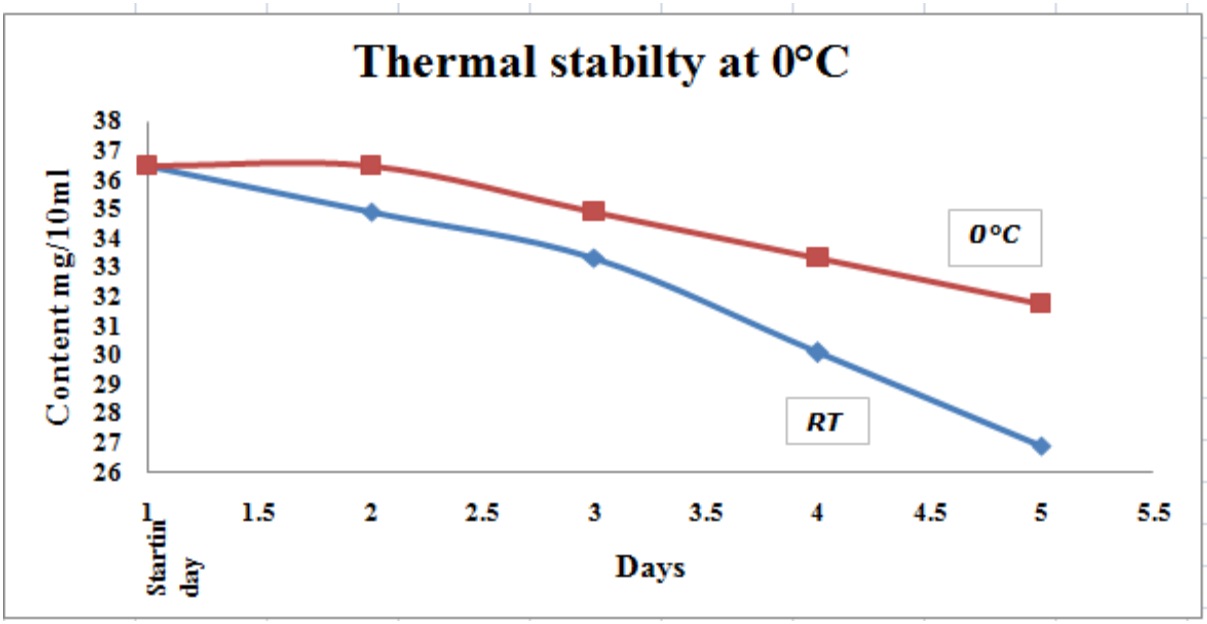


Figure 6: *Stability curve at 12°C*

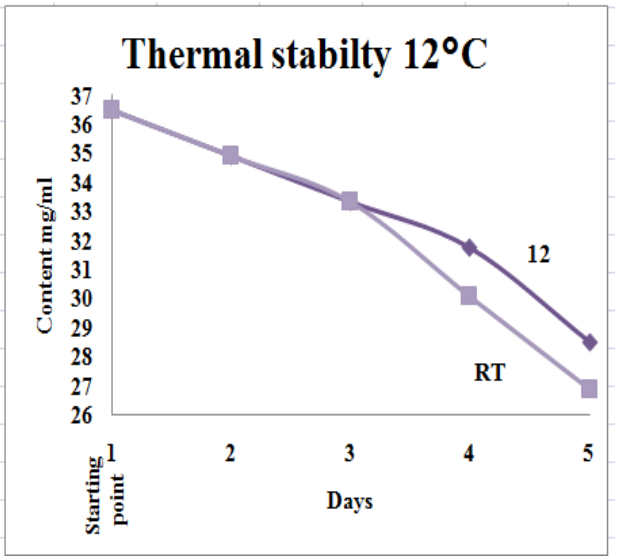
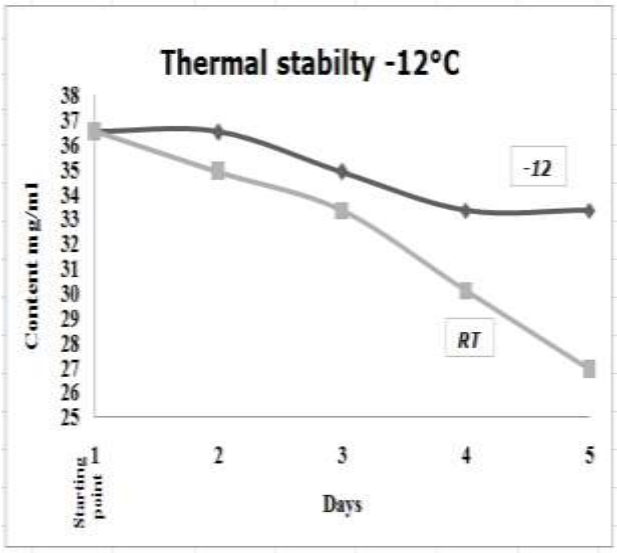


Figure 7:*Stability curve at -12°C*



Quantitation Of Vitamin C And Ph Treatments

Vita C itself has acidic pH (2.5 to 3.5). Its activity hypothetically decreases with increase in pH. Manipulation in pH is done by adding acid or base as per need in it. Stability of Vita C decreases normally so content varies with pH change such as in figure below [Fig 8]. Digital pH meter is used for measurement of variation.

Quantitation Of Vitamin C In Open Air

Air treatments effects the stability due to oxidation of vitamin, Open air or environmental stability is assessed by keeping the sample in glass amber colored bottle, in normal light area with no change in pH and at room temperature [Fig 9]. Available Vita C exposed to air in wide open mouth bottle for 240 minutes [4 hours] time duration.

Figure 8: *Stability curve at various ph*

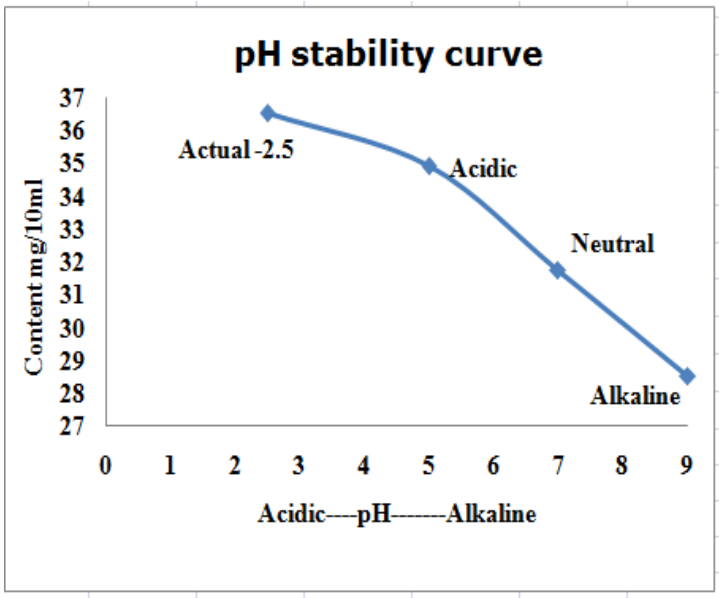
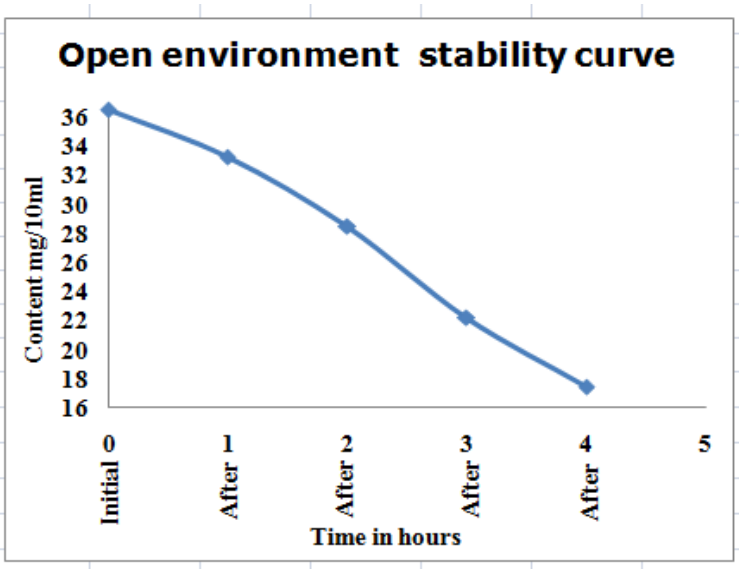


Figure 9: *Stability curve in open air*



RESULTS

Actual content of ascorbic acid in lemon juice without any hindrance was 36.5 mg/100ml

ESTIMATION OF VITAMIN C FROM FRESH LEMON JUICE OF DIFFERENT STAGES OF RIPENING [Table 3]

Age has significant effect on lemon juice content like high content 74.6 mg/100 ml was found in unripe stage of lemon whereas least amount 20.6 mg/100 ml is in stale lemon's juice. Estimation of lemon vitamin C content was done with the help of standard ascorbic acid titration as below [Table 2].

Table 2: *Standardization of Ascorbic acid [API]*

S. no	Volume of standard [ml]	Burette reading*		Volume of Iodine consumed/10 ml
		Initial reading	Final reading	
1	10 ml	0.0	6.5	6.5
2	10ml	6.5	12.9	6.4
3	10ml	13	18.2	6.2
Average				**19.1/3=6.3**

* *Average of triplicates; mg/ml: milligrams per milliliter*

Table 3: *Titration Vitamin C content variation in age of lemons*

S. no	Lemon type	Volume of lemon juice [ml]	Volume of iodine consumed*	Vitamin C mg/100ml* BR* Factor [10]
1	Unripe	10	4.7	74.6
2	Semi ripe	10	3.5	55.5
3	Ripe	10	2.3	36.5
4	Stale	10	1.3	20.6

* *Average of triplicates; mg/ml: milligrams per milliliter, BR: burette reading*

Effect Of Temperature On Contents Of Lemon [Table 4-11]

Thermal treatments have shown effect on lemon content [mg /100ml] after exposing the juice separately for different periods of time, at room temperature it was found at temperature 26.9, at slight higher temperature of 40°C, it was found 23.8, increasing up to 60°C, amount was found 22.22 but the highest temperature of 80°C, it was found very less 17.82 mg/100ml. In the opposite side of lower temperature content of lemon [mg/100ml] was found 21.9 at 12°C , 13.01 at 0°C and 8.68 at freezing temperature -12°C.

Table 4: *Stability of Vitamin C at Room Temperature*

S. no	Time interval [Hours]	Burette reading for 10 ml	Amount [mg/100 ml] BR* Factor [10]
1	Zero initial point [Day 1]	2.3	36.5
2	After 24 hours [Day 2]	2.2	34.9 After storage
3	After 48 hours [Day 3]	2.1	33.33
4	After 72 hours [Day 4]	1.9	30.1

Table 5: *Stability of Vitamin C at Temperature [40°C]*

S. no	Time interval [Hours]	Burette reading for 10 ml	Amount [mg/100 ml] BR* Factor [10]	
1	Zero initial point	2.3	36.5	
2	After 1 hour	2.2	34.9	After storage
3	After 2 hours	1.9	30.1	
4	After 3 hours	1.7	26.9	
5	After 4 hours	1.5	23.8	

Table 6: *Stability of Vitamin C at Higher Temperature [60°C]*

S. no	Time interval [Hours]	Burette reading for 10 ml	Amount [mg/100 ml] BR* Factor [10]	
1	Zero initial point	2.3	36.5	
2	After 1 hour	2.0	31.7	After storage
3	After 2 hours	1.9	30.1	
4	After 3 hours	1.6	25.4	
5	After 4 hours	1.4	22.22	

Table 7: *Stability of Vitamin C at Higher Temperature [80°C]*

S. no	Time interval Hours	Burette reading for 10 ml	Amount [mg/100 ml] BR* Factor [10]	
1	Zero initial point	2.3	36.5	
2	After 1 hour	2.0	31.7	After storage
3	After 2 hours	1.6	25.4	
4	After 3 hours	1.3	20.6	
5	After 4 hours	1.0	15.87	

Table 8: *Stability of Vitamin C lower temperature above zero [12]*

S. no	Time interval Hours	Burette reading for 10 ml	Amount [mg/100 ml] BR* Factor [10]	
1	Zero initial point [Day 1]	2.3	36.5	
2	After 24 hours [Day 2]	2.2	34.9	After storage
3	After 48 hours [Day 3]	2.1	33.33	
4	After 72 hours [Day 3]	2.0	31.75	
5	After 96 hours [Day 4]	1.8	28.5	

Table 9: *Stability of Vitamin C at freezing Temperature [0]*

S. no	Time interval Hours	Burette reading for 10 ml	Amount [mg/100 ml] BR* Factor [10]	
1	Zero initial point [Day 1]	2.3	36.5	
2	After 24 Hours[Day 2]	2.3	36.5	After storage
3	After 48 Hours [Day 3]	2.2	34.9	
4	After 72 Hours [Day 3]	2.1	33.33	
5	After 96 Hours [Day 4]	2.0	31.75	

Table 10: *Stability of Vitamin C lower temperature below zero [-12]*

S. no	Time interval Hours	Burette reading for 10 ml	Amount [mg/100 ml] BR* Factor [10]	
1	Zero initial point [Day 1]	2.3	36.5	
2	After 24 hours[Day 2]	2.3	36.5	After storage
3	After 48 hours [Day 3]	2.2	34.9	
4	After 72 hours [Day 3]	2.1	33.33	
5	After 96 hours [Day 4]	2.1	33.33	

Table 11: *Percent content loss with thermal treatments*

S.no	Temperature ± 2.5 °C	Amount mg /10 ml			Percent[%]loss [mg/10ml]
		Before treatment [W_i]	After treatment [W_f]	Loss [W_i - W_f]	
1.	25.0	36.5	26.90	9.6	26.3
2.	40.0	36.5	23.8	12.7	34.8
3.	60.0	36.5	22.22	14.28	39.12
4.	80.0	36.5	15.87	20.63	56.52
5.	12.0	36.5	28.5	8.00	21.9
6.	0.00	36.5	31.75	4.75	13.01
7.	-12.0	36.5	33.33	3.17	08.68

EFFECT OF PH TREATMENTS ON CONTENT OF LEMON

The effect of pH on active content of lemon juice was studied using acidulent and alkalizing agents like hydrochloric acid and sodium hydroxide in acidic ph of 5 it was found 34.9 mg/100ml but at alkaline oh of 9 it was decreased to 28.5 mg/100ml as shown below.
[Table 12]

Table 12:*Stability of Vitamin C at various pH*

S. no	Condition*	pH value ±0.5*	Burette reading for 10 ml	Amount [mg/100 ml] BR* Factor [10]	
1	Actual pH	2.5	2.3	36.5	
2	Acidic	5.0	2.2	34.9	After pH
3	Neutral	7.0	2.0	31.75	manipulation
4	Alkaline	9.0	1.8	28.5	

* pH adjusted by 0.1M NaOH and 0.1M HCl; measured after adding all reactants.

Effect of environment on content of lemon

Open air susceptibility was checked at RT on vitamin C [ripened lemon] for four hours of treatment it was found 17.6 mg/100 ml after 4 hours in air. Air has oxygen in it that can deteriorate it. [Table 13]

Table 13:*Open environment stability*

S. no	Time [hours]	Burette reading for 10 ml	Amount [mg/100 ml] BR* Factor [10]	
1	Zero[Initial]	2.3	36.5	
2	After 1	2.1	33.3	After storage air at RT*
3	After 2	1.8	28.5	
4	After 3	1.4	22.2	
5	After 4	1.1	17.46	

* RT: - Room temperature and normal humidity.

CONCLUSIONS

According to the above performed experimental based on classical methodology of titration, it has been revealed that the amounts of Vita C in lemon are influenced by the age of lemon, intensity of thermal treatments, exposure to air and pH manipulations. The growing age of the lemon decreases the Vita C concentration. The contents of vitamin C deteriorate but a very less amount was found to be degraded at room temperature [27±2 °C] in closed container in normal light and humidity. As the thermal treatment get severe and rises to higher side the content diminishes quickly with wide range as compared to RT. Another aspect of thermal treatment is storage of lemon juice at low temperature; it allows in sustaining the content for longer time in days. It is better to store the ascorbic acid in lower temperature range [-12±2 °C to 12±2 °C]. Percent loss of Vitamin C at high temperature [80±2 °C] is more than double the loss at normal temperature but at freezing temperature [0±2 °C]; the loss is approximately half as compared to normal temperature. When the temperature is kept below the freezing point, the contents of lemon juice get degraded if stored for longer time. Air and pH can also lead to decrease in amount of the Vitamin C. It is good to store the juice containing Vita C at normal pH as because alkaline pH diminishes its amount. Air treatments can also lead to deterioration of the contents due to severe oxidation of Vita C into dehydrate derivatives which are not in active form, so it is mandatory to store it in cool ,dry and light protective well closed container with the addition of preservatives and pH stabilizing agents.

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