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Stability Comparison of Vitamin C (Ascorbic Acid) In Freshly Prepared Fruit Juice and Marketed Formulation of Strawberry

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ABSTRACT

Vitamin C is an essential nutrient needed for maintaining for human health. Strawberry juice is easily damaged in transit due to temperature fluctuations and in the presence of oxygen in an oxidation reaction that may significantly reduce shelf life. In this way, one week stability studies of freshly prepared juice of strawberry and the marketed formulation of strawberry (juicy jelly) was conducted and the amount of ascorbic acid degraded, juice kept at room temperature as well as 0-6°C and degraded amount was found by iodine titration method. The degradation of vitamin C was found in freshly prepared juice and marketed formulation that was kept at room temperature. Furthermore, it showed fast decrease of shelf life for freshly prepared juice kept at room temperature compared to the freshly prepared juice kept at refrigerator temperature and marketed formulation. The degradation amount of vitamin c in freshly prepared juice was found to be more compared to the marketed formulation. For freshly prepared juice at room temperature vitamin C degradation rate ranges from 240.677mg\L to 166.10mg\L. At refrigerator condition the vitamin C content ranges from 240.67mg\L to 176.27mg\L. For marketed formulation kept at room temperature the vitamin C content ranges from 50.8mg/L to 41.2mg/L. At refrigerator condition the vitamin C content ranges from 50.8mg/L to 48.6mg/L.

Keywords: Vitamin C (Ascorbic acid), Strawberry juice, Marketed Formulation (juicy jelly), Iodine solution, Starch solution, Room Temperature, Refrigerator.

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INTRODUCTION

Strawberries one of the healthiest fruits you can eat. They contain high levels of nitrate and good source of potassium, which can help to increase blood and oxygen flow to the muscles. The ellagic acid and other phytochemicals found in strawberries can help fight bad cholesterol by counteracting the negative effects of LDL, and they also have an anti-inflammatory effect and may reduce blood lipids¹.

The causes for the red color of strawberry are called "anthocyanins". There are two main anthocyanin's that are responsible for the color of strawberries, namely, pelargonin (pelargonidin-3-glucoside) and cyanin (cyanidin-3-monoglucoside). Pelargonin accounts for a minimum of nearly two-thirds of a strawberry's anthocyanin pigments. Anthocyanins are very powerful absorbers of the single, ionized oxygen atoms commonly referred to as "free radicals". It is therefore speculated that these same anthocyanin's may have similarly strong antioxidant capabilities in vivo (that is, inside the body)².

Strawberries (**Fragaria X ananassa**), ORAC Value 3577, Nutrition Value per 100 g. (Source: USDA National Nutrient data base)³. Nutritional values of strawberry are mentioned in table 1

Table 1: Nutritional values of strawberry

Principle	Nutrient Value	Percentage of RDA
Energy	32 Kcal	1.5%
Carbohydrates	7.7 g	6%
Protein	0.67 g	0.1%
Total Fat	0.30 g	1%
Cholesterol	0 mg	0%
Dietary Fiber	2.0 g	5%
Vitamins		
Folates	24 µg	6%
Niacin	0.386 mg	2.5%
Pantothenic acid	0.125 mg	2.5%
Pyridoxine	0.047 mg	3.5%
Riboflavin	0.022 mg	2%
Vitamin A	12 IU	0.5%
Vitamin C	58.8 mg	98%
Vitamin E	0.29 mg	2%
Vitamin K	2.2 µg	2%
Electrolytes		
Sodium	1 mg	0%
Potassium	153 mg	3%
Minerals		
Calcium	16 mg	1.6%
Iron	0.41 mg	5%

Magnesium	13 mg	3%
Manganese	0.386 mg	17%
Zinc	0.14 mg	1%
Phytonutrients		
Carotene-β	7 μg	--
Lutein-zeaxanthin	26 μg	--

Red color of strawberry easily changes upon storage and is replaced by a dull brownish color (Gössinger *et al.*, 2009)⁴, This is the result of the simultaneous degradation of natural red anthocyanin pigments to colorless compounds (Kirca & Cemeroğlu, 2003; Mercadante and Bobbio, 2008)⁵ and the formation of brown pigments due to enzymatic and/or non-enzymatic reactions (Bharate and Bharate, 2014; Garzón and Wrolstad, 2002; Gössinger *et al.*, 2009)⁶.

Role of Vitamin C (Ascorbic acid)

Vitamin C (Ascorbic acid), also known as L-ascorbic acid, is a water-soluble vitamin. Vitamin C, or Ascorbic acid is a cofactor. Cofactors are the most important components required to maintain fundamental processes throughout the body. Basic nervous system functions such as neurotransmitter synthesis and healthy cell-to-cell communication would not be possible without the presence of necessary vitamin, mineral and amino acid cofactors. Supplementing a balanced diet with Vitamin C has been clinically shown to promote healthy nervous system function. Because of its nutritive value⁷. Not only does vitamin C support the immune system, it also acts as an antioxidant, keeping your cells healthy. It also creates collagen an essential component needed for wound healing⁸.

Vitamin C Deficiency Symptoms⁹

A severe vitamin C deficiency will result in scurvy, a disease resulting from the breakdown of collagen. Scurvy will make feel fatigued and lethargic. It affects bone and muscle strength and it stifles the immune system.

- Easy bruising
- Swollen gums
- Bleeding gums
- Slow wound healing
- Gingivitis (inflammation of the gums)
- Dry and splitting hair
- Dry red spots on the skin
- Rough, dry, scaly skin

- Nosebleeds
- Weakened immune system
- Digestive disorders like leaky disease
- Possible weight gain because of slowed metabolism
- Swollen and painful joints

Health problems related to a vitamin C deficiency can get much worse over time, and may lead to some serious health issues. Long Term Problems from Low Levels of Vitamin C include:

- High blood pressure
- Gallbladder disease
- Stroke
- Certain cancers
- Atherosclerosis

In plants, vitamin C is a substrate for ascorbate peroxidase. This enzyme utilizes ascorbate to neutralize toxic hydrogen peroxide (H₂O₂) by converting it to water (H₂O)¹⁰.

Recommended Intakes¹¹

Intake recommendations for Vitamin C (Ascorbic acid) and other nutrients are provided in the Dietary Reference Intakes (DRIs) developed by the Food and Nutrition Board (FNB) at the Institute of Medicine (IOM) of the National Academies (formerly National Academy of Sciences).

Table 2 : Recommended dietary allowances of Vitamin C (Ascorbic acid)

Age	Male	Female	Pregnancy	Lactation
0-6 months	40 mg*	40 mg*		
7-12 months	50 mg*	50 mg*		
1-3 years	15 mg	15 mg		
4-8 years	25 mg	25 mg		
9-13 years	45 mg	45 mg		
14-18 years	75 mg	65 mg	80 mg	115 mg
19+ years	90 mg	75 mg	85 mg	120 mg

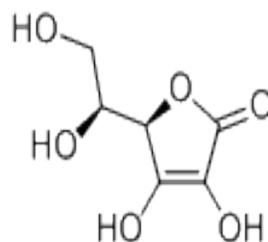
Smokers Individuals who smoke require 35 mg/day more vitamin C than nonsmokers.

* Adequate Intake (AI)

Strawberries are an excellent source of vitamin C, One serving (about half a cup) of strawberries contains roughly half your daily dose of vitamin C, which can boost immunity, improve eye health, and keep your skin looking younger. Vitamin C is also an anti-oxidant, which may help to prevent certain cancers and inflammation¹².

Properties of Vitamin C (Ascorbic acid)^{13,14}**Description:**

It is a six carbon compound related to glucose. It is found naturally in citrus fruits and many Vegetables. Ascorbic acid is an essential nutrient in human diets, and necessary to maintain connective tissue and bone. Its biologically active form, vitamin C, functions as a reducing agent and coenzyme in several metabolic pathways. Vitamin C is considered an antioxidant.

Structure:

Chemical Formula: C₆H₈O₆

Molecular Weight: 176 gm/mol

IUPAC Name : (5R)-5-[(1S)-1, 2-dihydroxyethyl]-3, 4-dihydroxy-2, 5-dihydrofuran-2-one.

Appearance: white to slightly yellowish crystalline powder, practically odorless, with Pleasant, sharp, strong acidic taste.

Boiling point: 553 °C

Melting point: 190-194 °C (dec.)

Storage temperature: 0-6°C

Solubility: Water Solubility: 333 g/L (20 °C)

Insoluble in ether, chloroform, benzene, petroleum ether, oils, fats, fat solvents

PH: 1.0 - 2.5 (25°C, 176g/L in water)

Stability: Stable. May be weakly light or air sensitive. Incompatible with oxidizing agents, alkalis, iron, copper.

MATERIALS AND METHOD:**Preparation of juice**

Fresh strawberry fruits were purchased from market then Wash strawberries and remove stem and cut into small pieces. Transfer these strawberry pieces to a blender, Blend until smooth puree is formed. Add water and blend again for a minute. Place a fine mesh over a large container and pour prepared puree over it to remove the foam results during blending.

Marketed formulation

Marketed formulation (Juicy jelly) was purchased from the market

Juice storage

Freshly prepared juice and juicy jelly of strawberry were divided into two equal portions and kept in room temperature and at refrigerator (2-8 °C).

Vitamin C (Ascorbic acid) determination¹⁵

Method: Iodine titration method

Preparation of reagents:**a) 0.5% Starch Indicator Solution**

1. Add approximately 0.5 g soluble starch to 50 ml near-boiling deionized water.
2. Mix well and allow to cool before use.

b) Iodine Solution

1. Dissolve 5.00 g potassium iodide (KI) and 0.268 g potassium iodate (KIO₃) in 200 mL of distilled water.
2. Add 30 mL of 3 M sulfuric acid.
3. Pour this solution into a 500 mL graduated cylinder and dilute it to a final volume of 500 mL with distilled water.
4. Mix the solution.
5. Transfer the solution to a 600 mL beaker. Label the beaker as your iodine solution.

c) Vitamin C Standard Solution

1. Weigh out accurately about 0.250 g vitamin C (ascorbic acid) into a 250 mL volumetric flask. Add about 100 mL distilled water and swirl to dissolve.
2. Add distilled water to make up to the mark. Label the flask as your vitamin C standard solution.

Standardizing the Iodine Solution

1. Using a pipette, add 25.00 mL aliquot of vitamin C standard solution to a 125 mL conical flask. Note: this would contain 0.0250 g of vitamin C.
2. Add 10 drops of 0.5% starch solution.
3. Rinse your burette with a small volume of the iodine solution and then fill it. Record the initial volume.
4. Titrate the solution until the endpoint is reached. This will be when you see the first sign of blue color that persists after 20 seconds of swirling the solution.
5. Record the final volume of iodine solution. The volume that was required is the starting volume minus the final volume.
6. Repeat the titration at least twice more. The results should agree within 0.1 ml.

Titration Juice Samples

1. Add 25.00 ml of juice sample to a 250 ml conical flask.

Titrate against iodine solution in the burette until the endpoint is reached. It is when you get a color that persists longer than 20 seconds.

2. Repeat the titration until you have at least three titers that agree to within 0.1 ml. Note: if the titers are too big (>25 mL) consider cutting back the aliquot (sample) of strawberry juice to 10 ml or less.

RESULTS AND DISCUSSION

One week stability studies of Vitamin C (Ascorbic acid) at room temperature and refrigerator (2-8°C) was conducted for both, freshly prepared juice as well as for marketed formulation and amount of Vitamin C (Ascorbic acid) was determined by Iodine titration method and results were mentioned in the table no.3,4 and 5,6.



Figure 1: Left side fresh marketed formulation, middle after eight days in refrigerator (2-8°C), Right side after eight days at room temperature (25°C).



Figure 2: Left side freshly prepared juice, middle juice after one week at refrigerator temperature (2-8°C), Right side juice after one week at room temperature (25°C).



Figure 3: Left side End point (blue) of Vitamin C standard solution, Middle one is the end point of marketed formulation and Right side end point (dark brown) of fresh juice.

Table 3: Degradation of vitamin C at room temperature (25°C) and status of freshly prepared strawberry juice

S.No	Temperature	Sampling (days)	Status of juice	End point	Amount of vitamin C (mg/1000ml)	Degradation rate of Vitamin C
1	Room Temperature	1 st day	Bright red color and fresh	Dark brown color	240.677	0.00
2		2 nd day	Bright red color and fresh	Dark brown color	230.508	10.17
3		3 rd day	Bright red color and slight change in aroma	Dark brown color	223.728	16.95
4		4 th day	red color and aroma changed	Dark brown color	196.610	44.06
5		5 th day	red color and change in aroma	Dark brown color	186.440	54.23
6		6 th day	Color of juice turned to light and decomposed	Dark brown color	179.66	61.01
7		7 th day	Color of juice reduced and decomposed	Dark brown color	172.88	67.79
8		8 th day	Turned to dull red color and completely decomposed	Dark brown color	166.10	74.57

Table 4: Degradation of vitamin c at refrigerated temperature (0 -8°C) and status of freshly prepared juice

S.No	Temperature	Sampling (days)	Status of juice	End point	Amount of vitamin c (mg/1000ml)	Degradation rate of Vitamin C
1	Refrigerator	1 st day	Bright red color and fresh	Dark brown color	240.67	0.00
2		2 nd day	Bright red color and fresh	Dark brown color	237.28	3.39
3		3 rd day	Bright red color and fresh	Dark brown color	227.11	13.57
4		4 th day	Bright red color and fresh	Dark brown color	210.16	30.51
5		5 th day	Bright red color and fresh	Dark brown color	200	40.67
6		6 th day	Bright red color and fresh	Dark brown color	193.22	47.45
7		7 th day	Bright red color and fresh	Dark brown color	183.05	57.62
8		8 th day	Bright red color and fresh	Dark brown color	176.27	64.40

Table 5: Degradation of vitamin C at refrigerator(0-8°C) and status of marketed formulation

S.No	Temperature	Sampling (Days)	Status Of Juice	End Point	Amount Of Vitamin C (Mg/1000ml)	Degradation rate of Vitamin C
1	Refrigerator	1 st day	Bright pink color and fresh	Orange color	50.8	0.00
2		2 nd day	Bright pink color and fresh	Orange color	50.8	0.00
3		3 rd day	Bright pink color and fresh	Orange color	50.8	0.00
4		4 th day	Bright pink color and fresh	Orange color	49.8	1.00
5		5 th day	Bright pink color and fresh	Orange color	49.6	1.20

6	6 th day	Bright pink color and fresh	Orange color	49.2	1.60
7	7 th day	Bright pink color and fresh	Orange color	48.8	2.00
8	8 th day	Bright pink color and fresh	Orange color	48.6	2.20

Table 6: Degradation of vitamin C at room temperature(25°c) and status of marketed formulation

S.No	Temperature	Sampling (Days)	Status of Juice	End Point	Amount Of Vitamin C (Mg/1000ml)	Degradation rate of Vitamin C
1	Room temperature	1 st day	Bright pink color and fresh	Orange color	50.8	0.00
2		2 nd day	Bright pink color and fresh	Orange color	46.8	4.00
3		3 rd day	Bright pink color and fresh	Orange color	44.2	6.60
4		4 th day	Bright pink color and fresh	Orange color	43.2	7.60
5		5 th day	Bright pink color and fresh	Orange color	42.6	8.20
6		6 th day	Bright pink color and fresh	Orange color	42	8.80
7		7 th day	Bright pink color and fresh	Orange color	41.6	9.20
8		8 th day	Bright pink color and fresh	Orange color	41.2	9.60

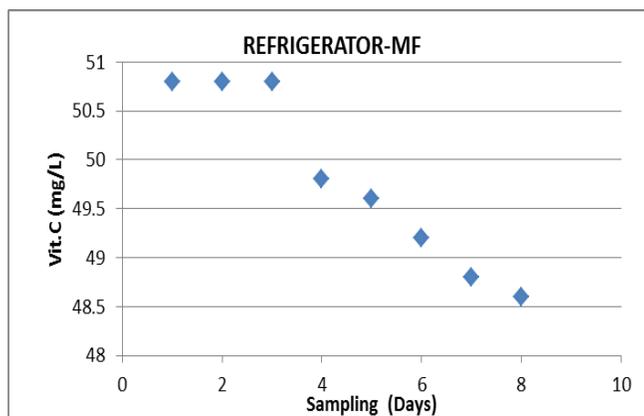


Figure 4: Degradation of Vitamin C (Ascorbic acid) in marketed formulation at refrigerator (2-8°C) temperature.

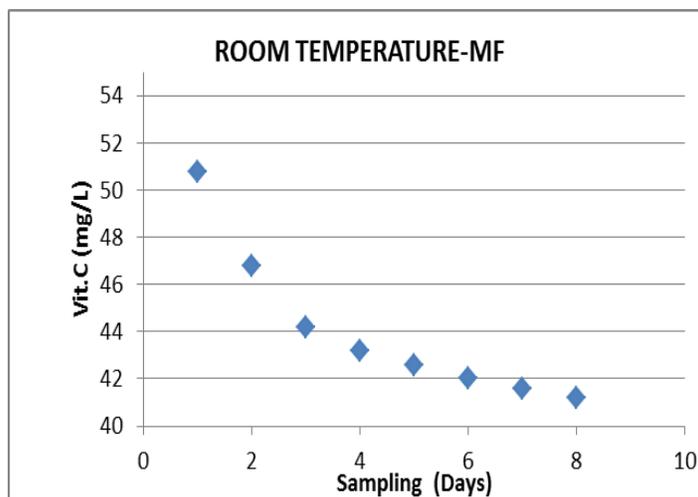


Figure 5: Degradation of Vitamin C (Ascorbic acid) in marketed formulation at room temperature (25°C)

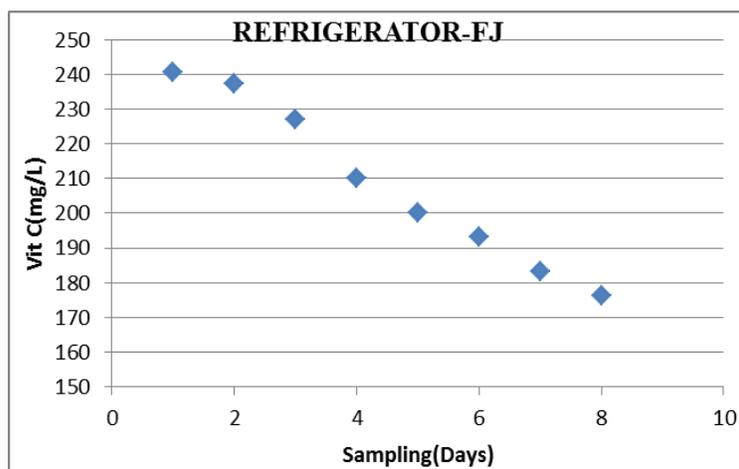


Figure 6: Degradation of Vitamin C (Ascorbic acid) in freshly prepared juice at refrigerator (2-8°C) temperature.

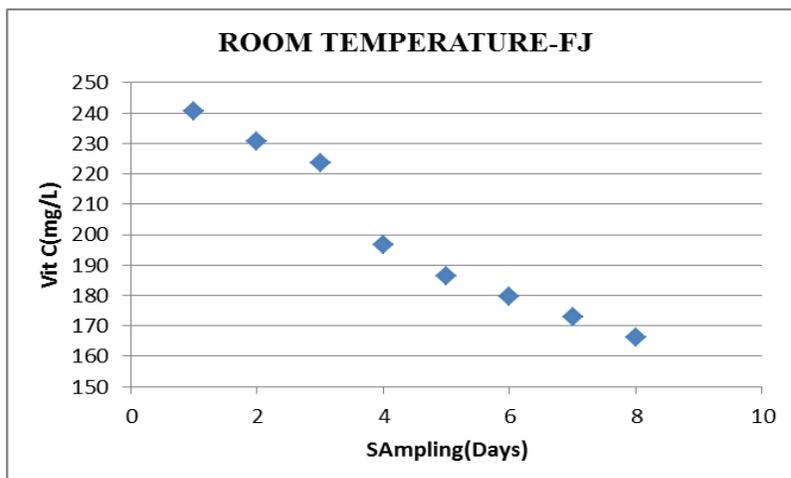


Figure 7: Degradation of Vitamin C (Ascorbic acid) in freshly prepared juice at room temperature (25°C)

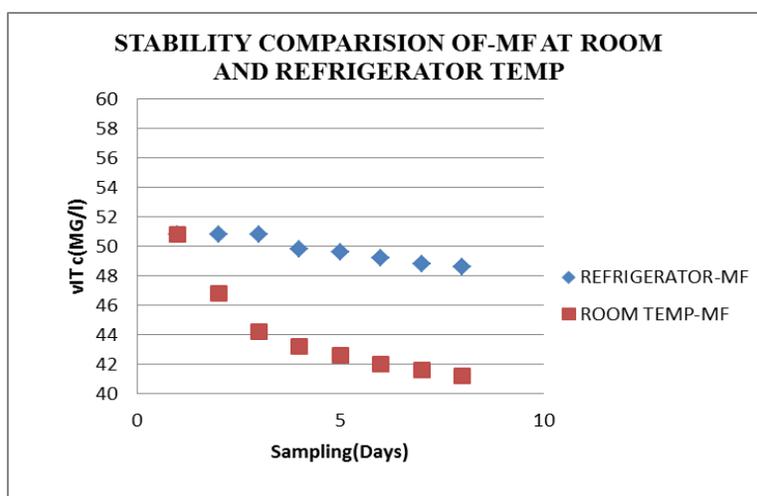


Figure 8: Stability comparison of marketed formulation of strawberry at room temperature and refrigerator temperature

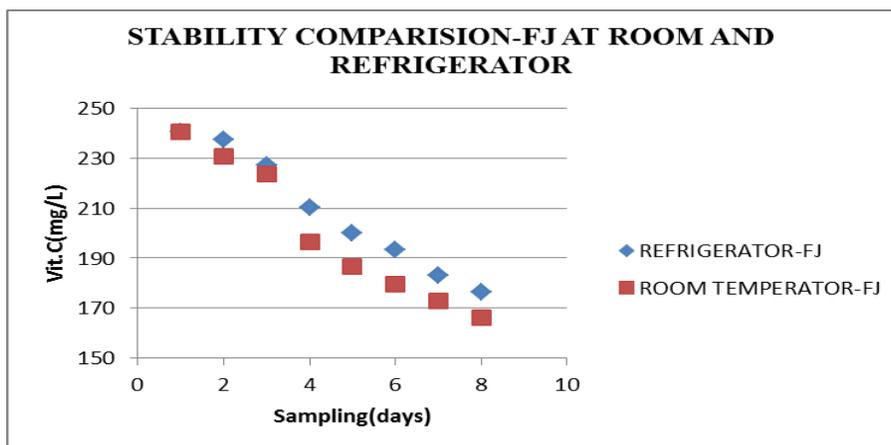


Figure 9: Stability comparison of freshly prepared juice (FJ) of strawberry at room temperature and refrigerator temperature

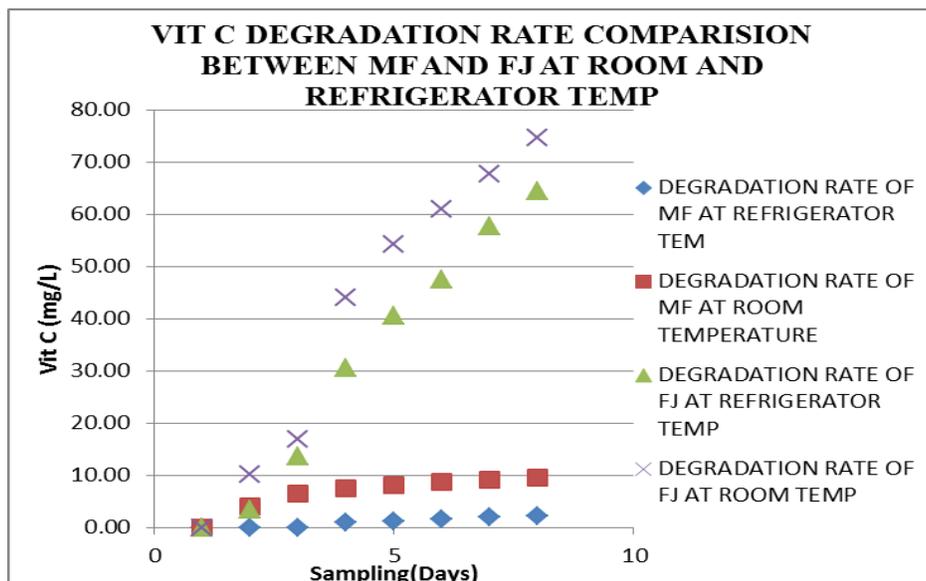


Figure 10: Comparison of Vitamin C Degradation rate between marketed formulation and freshly prepared juice at room temperature and refrigerator temperature.

SUMMARY:

On first day the vitamin C contents of fresh juices (kept at room temperature (25°C) and refrigerator (2-8°C) temperature) were found to be 240.677 mg/L, in the same manner the vitamin C contents of Marketed formulation(MF)(juicy jelly) (kept at room temperature (25°C) and refrigerator (2-8°C) temperature) were found to be 50.8 mg/L . During the period of one week, the vitamin C content was found significantly reduced at room temperature condition and at refrigeration temperature conditions in fresh juice (FJ) and Marketed formulation (MF). At room temperature, the vitamin C content ranges from 240.677 mg/L to 166.10 mg/L, At refrigeration condition, the vitamin C content ranges from 240.677 mg/ L to 176.27 mg/L of fresh juice(FJ). Degradation values of FJ were mentioned in table no.3, 4.

At room temperature, the vitamin C content ranges from 50.8 mg/L to 41.2 mg/L, At refrigeration condition, the vitamin C content ranges from 50.8 mg/ L to 48.6 mg/L of marketed formulation(MF). Degradation values of MF were mentioned in table no.5, 6.

Usually the end point of vitamin C standard solution is blue color by iodine titration method. Due to the color pigments in fresh juice the end point was dark brown color and due to the color interference in marketed formulation the end point was orange color. We can observe the color at the end point of standard solution, MF and FJ in “Figure No. 3”. After 2 days of storage the color of the juice kept at room temperature gets started changing to red color finally to light red color after one week, no color change was observed to the juice kept at refrigerator temperature. The MF

kept at room and refrigerator temperature maintained the color throughout the week. The color status of the MF and FJ shown in figure 1 and 2.

CONCLUSION:

The results obtained after an accurate analysis and precise measurement shows that the vitamin C contents reduced in both freshly prepared juices and marketed formulation kept at room temperature and refrigerated temperature. May be due to the absence of preservatives in FJ Vitamin C contents reduced rapidly compared to MF. By observing degradation values mentioned in the table No's 3, 4 and 5, 6 refrigeration storage is more preferable due to the slow degradation rate in FJ and MF as compared to Room storage as per analytical data mentioned. Degradation is more rapid in the juice kept at room temperature in FJ and MF when compared to juice kept at refrigerator temperature and more over the juice kept in refrigerator is fresh and has fresh aroma of strawberry in FJ and MF. FJ is preferable than MF because in FJ the vitamin C content is very high compared to MF. FJ can be used for two days kept at room temperature and can be used one week kept at refrigerator temperature.

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