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Determination of Amount of Ascorbic Acid in Selected Citrus Fruit Varieties in Luwero District, Uganda

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Abstract: **The study was to determine the concentration of ascorbic acid among the citrus fruits collected from Luwero District so as to find out the quantity of citrus fruits needed to provide recommended daily allowance of ascorbic acid for healthy eating. The study took experimental design. The ascorbic acid content of the juices of four different citrus fruits– Exotic Navel orange, Local Valencia orange, Local Mandarin and Local Kaffir lime was determined iodometrically in order to identify which fruit would best supply the ascorbic acid needed for the body and at what quantity. Results showed that Exotic Navel orange had the highest value of ascorbic acid, 23.37mg/100g, followed by Local Valencia orange, Local Mandarin and Local Kaffir Lime with ascorbic acid concentration of 21.63mg/100g, 20.04mg/100g and 12.01mg/100g respectively. It therefore follows that Exotic Navel orange would supply more ascorbic acid for body need compared to the other three fruits. The quantity of each citrus fruit needed to provide recommended daily allowance of ascorbic acid, 45mg for an adult person, was also determined. For example, it was recommended that consuming one and a quarter of Local Valencia orange every day is enough for an adult person to get the daily recommended amount of ascorbic acid to prevent non – communicable diseases. Taking two and half pieces of Local Mandarin would supply 45 mg of ascorbic acid required for daily need. And consuming one Exotic orange every day is enough for an adult to get recommended amount of ascorbic acid daily. However, about two and a quarter Local Kaffir lime will have to be eaten to meet the same daily requirement of vitamin C for an adult person.**

Keywords: **Citrus Fruit, Ascorbic Acid, Exotic Navel Orange.**

1. INTRODUCTION

Ascorbic acid primarily comes in two forms – L-ascorbic acid and D-ascorbic acid. The L variety, which can come in both natural (found in fruits and vegetables) and synthetic forms (found in most other supplements), is synonymous with vitamin C and carries all its benefits, while the D carries identical antioxidant properties but not the vitamin C content (Diane, 2014).Unlike vitamin D, human cannot synthesize vitamin C. More than 90% of the vitamin C in human diets is supplied by fruits and vegetables (Alibone, 2000). Because cooking destroys the vitamin (Nagy, 1980), so raw citrus fruits and their juices are the main source of ascorbic acid for most people. For example an average orange of 131g will have approximately 70 mg of vitamin C while the average lemon will have around 46 mg (Levine *et al.,* 1999). Most of these fruits are locally found in Luwero District.

Consumption of citrus juice is found to be beneficial. For example, Vitamin C is an essential nutrient that plays a vital role in protecting the body from infections and diseases. It is needed for the formation of collagen, the protein that makes up connective tissue, and is essential to muscles, bones cartilages, blood vessels, tissues, skin and teeth. It also

functions in absorption of inorganic iron, reduction of plasma cholesterol level, enhancement of the immune system, and reaction with singlet oxygen and other free radicals. As an antioxidant, it is reported to reduce the risk of arteriosclerosis, cardiovascular diseases, infectious diseases, asthma, cataract, Diabetes Mellitus and some forms of cancer (Abd-Ghafar *et al*., 2010). Lack of vitamin C in the diet causes the deficiency disease scurvy. It may also result in hemorrhages under the skin, poor wound healing, edema and weakness (Enloe *et al*., 1985).

Recommended daily intake (RDI) of vitamin C, according to World Health Organization (WHO) for healthy living, is 45 mg for an adult person per day, regardless of gender(WHO, 2004).The RDI of vitamin C recommended for East African Countries including Uganda is 45mg for adult males and females (East African Community, 2013).

When addressing journalists during training on proper dissemination of information on Non Communicable Diseases (NCDs) in December 2017, the director of Makerere University Lung Institute, Dr. Bruce Kirega said NCDs such as cancer, diabetes, arteriosclerosis and cardiovascular diseases account for 40% of all death each year in Uganda. He reported that majority of Ugandan do not consume fruits and vegetables to the recommended level which could reduce the risk of NCDs (Tuhereza, 2017).

Although citrus fruits are the best sources of vitamin C most of the Luwero community members, just like other Ugandan, do not have enough knowledge about the quantity of citrus fruit needed to be consumed per day to provide the recommended daily allowance of vitamin C for a healthy living. There is also lack of coordinated determination of level of ascorbic acid in the varieties of citrus fruits found in Luwero District. Therefore the community is at high risk to NCDs. NCDs are largely preventable diseases. Beyond the appropriate medical treatment for those already affected, the public health approach of primary prevention is considered to be the most cost-effective, affordable and sustainable course of action to cope with the NCDs worldwide (WHO, 2003). Therefore it was necessary to determine the concentration of vitamin C in varieties of citrus fruits in Luwero District.

People in Luwero District eat citrus fruits without considering recommended daily allowance of Vitamin C (45mg/day for adult person). This is due to lack of coordinated investigation of the levels of Ascorbic acid in varieties of citrus fruits available in the district. So many people in the district are at high risk of lives treating non communicable diseases such as cancer, asthma, diabetes, arteriosclerosis and cardiovascular diseases which could be prevented by eating a right quantity of the citrus fruits that contain the recommended daily allowance of vitamin C. Therefore, the researcher wanted to determine the ascorbic acid content of selected local varieties and exotic citrus fruits available in Luwero District. Knowledge of the ascorbic acid content of citrus fruits would be used to give recommendations on the right quantity of citrus fruits to be eaten per person per day for prevention of non-communicable diseases. It would also be used to advise the farmers to plant citrus fruits with high amount of ascorbic acid.

2. METHODS AND MATERIALS

The study was carried out in Bugema University Bio - Chemistry Laboratory. Bugema University lies along Gayaza – Zirobwe road 32 km North of Kampala City, in Kalagala Sub-county Bamunanika County, Luwero District in Uganda. The coordinate of Bugema University are 0º34’10.48”N, 32º38’30.55”E. (Latitude: 0.57; Longitude 32.6418). The samples for experimentation were collected from the farmers within Luwero District.

In this study, experimental design method was used to determine the concentration of Vitamin C in freshly prepared fruit juice samples by iodometric titration. During this titration, a titrant of known concentration (iodine) reacted with a solution of the analyte of unknown concentration (vitamin C in fruit juice). Using a calibrated burette the exact amount of the titrant consumed was determined at the endpoint. The endpoint is the point at which the titration is complete, as determined by the colour change of an indicator. Titration method is used because it is accurate and precise method to determine vitamin C concentration in fruits. Samples of varieties of citrus fruits were used during the titration process. Experiments were carried on sixteen fruits of four varieties of citrus fruits collected from two different locations within Luwero District. The results of the amount of ascorbic acid calculated from the experimental results.

The target population were the community of Luwero District since only the concentration of citrus fruits found in the district were iodometrically determined so as to provide recommendation on the right consumption of the quantity of citrus fruits for prevention of non-communicable diseases.

The study involved sixteen pieces of citrus fruits selected through random sampling from four varieties of citrus fruits found in Luwero District.

Sample Collection:

Some fresh halfway ripen Seedless Exotic Naval oranges (*Citrus sinensis*), Local Valencia (*Citrus sinensis)*, Local Mandarin Oranges (*Citrus reticulate*) and Local Kaffir Lime (*Citrus hystrix*) citrus fruits were obtained from their respective trees, from the farmers at different areas(Mazzi and Vumba villages) within Luwero District, for the study. This was to enable the researcher extract more juice from the fresh halfway ripe fruits and also most people prefer halfway ripe oranges to raw one.

Research Instrument:

Laboratory apparatus and chemicals were used in this study to obtain the data. These apparatus and chemicals are listed as followed:

Materials:

Exotic Navel oranges (*Citrus sinensis*), Local Valencia (*Citrus sinensis*), Local Mandarin/tangerine Oranges (*Citrus reticulate*), Local Kaffir Lime (*Citrus hystrix*), Distilled Water (3000ml), Soluble starch (10 g), Solid KIO_3 (10 g), Solid KI (25g), 1 M H_2SO_4 (150 cm^3), 3M H_2SO_4

Apparatus:

Titration Set (Burette, Stand, Clamp, Tile and funnel), 250ml Conical Flask , Buchner Funnel, cheesecloth, 20ml Pipette, 25ml Measuring Cylinder, 250ml, Beaker, A knife, A cutting board, 100 ml graduated cylinder, 500 ml graduated, cylinder, filter papers, spatula and digital weighing balance.

Data Collection Method/ Procedures:

The samples of citrus fruits from Mazzi and Vumba villages in Luwero District were collected and practical experiments were carried out in the Chemistry Laboratory of Bugema to determine the amount of ascorbic acid in the citrus fruits. A number of experiments were carried out as illustrated in the parts below:

PART A: Preparation of sample fruit juices from the different citrus fruits:

One piece of fruit from each variety of citrus fruits was weighed, peeled and crushed with a machine (an industrial press) to get all the juice into pre-washed beakers. The juices obtained were filtered through cheesecloth (clean cotton cloth) to remove any pulps and seeds and the volume of each fruit juice were measured using $100cm^3$ measuring cylinder. After that each juice was made up to $100cm^3$ with distilled water and transferred back to the beaker. This formed sample 1 of the fruits varieties which were used for titration. The same procedure was used to prepare fruits juices of samples 2, 3 and 4.The mass of the fruit and volume of the juice from each fruit were recorded. Ascorbic acid is susceptible to oxidation by atmospheric oxygen over time. For this reason, the samples were prepared immediately before the titrations. The mass of the fruit and the volume of its juice were used for the calculation of concentration of ascorbic acid in each citrus fruit.

PART B: Preparation of 1% Starch Indicator Solution.

Distilled water (200 ml) was heated in a beaker on the stove to a temperature of about 80ºC and soluble starch (2g) was added to it. The solution was then stirred for 15 minutes to dissolve the starch and then the beaker removed from the burner and the solution allowed to cool (Ghani *et al*., 2016).

PART C: Preparation of Standard Iodine Solution.

A standard iodine solution was prepared by dissolving dried Potassium iodate, KIO_3 (0.26g) and potassium iodide, KI (5.00g) in some distilled water in a$500.00cm^3$ volumetric flask. The KI was in excess. The solution was acidified by adding 3M sulphuric acid (30.00ml). The mixture was swirled and the volume of solution made to 500.00ml with distilled water. The flask was stoppered and shaken to ensure homogeneity of content. The equation of the reaction:

$$IO_3^- + 5I^- + 6H^+ \longrightarrow 3I_2 + 3H_2O$$

This balanced equation was used to calculate the concentration of iodine in the solution (Izuagie and Izuagie, 2007).

PART D: Determination of the vitamin C concentration in fruit juices.

Ascorbic acid concentration was determined according to the method of (Nweze *et al*. 2015).The prepared sample juice (20ml) was pipetted and transferred into 250.00ml conical flask and then followed by 1% starch solution (10 drops) and

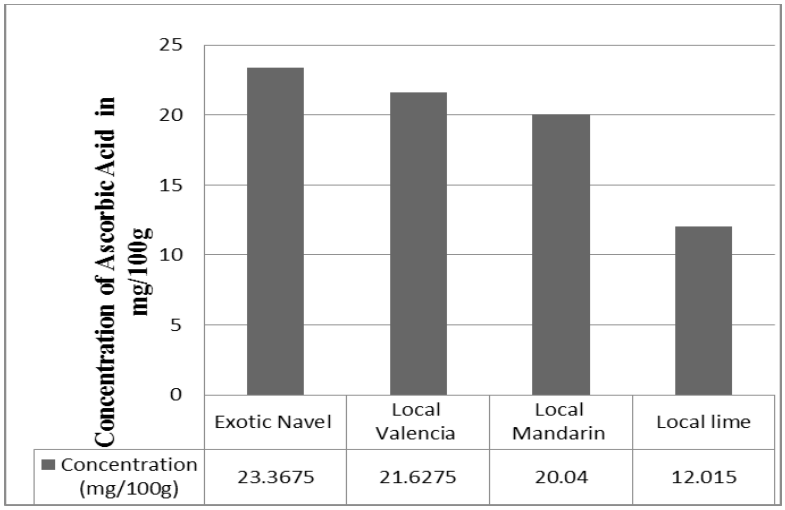
1M H_2SO_4(10.00 ml). It was then titrated with the standard iodine solution. The iodine oxidised the ascorbic acid to dehydro ascorbic acid as the iodine was reduced to iodide ions. Once all the ascorbic acid was oxidised, the excess iodine reacted with the starch indicator, forming a blue-black starch-iodine complex that persisted after 20 seconds of swirling the solution. This was the endpoint of the titration. Then the burette reading noted. The titration was repeated for three times, (Bekele and Geleta 2015). The results were recorded. The amount of ascorbic acid was calculated using mole ratio from the reaction equation: $C_6H_8O_6 + I_2 \longrightarrow 2I^- + C_6H_6O_6 + 2H^+$

This procedure was followed for determining the amount of ascorbic acid in all the samples of the citrus fruits.

The Analysis of Data: The quantitative data collected through the iodometric titration of citrus fruits juices was analyzed through calculation of concentration of ascorbic acid in the samples. One-way Completely Randomized Analysis of Variance (ANOVA) and Microsoft Office Excel were also used for further analysis of the results.

3. RESULT AND DISCUSSION

The amount of ascorbic acid in the samples of the varieties of citrus fruit was experimentally determined by Iodometric titration, using standard iodine solution of concentration 7.51 $X10^{-3}$M**.** The average concentration of each variety of citrus fruit was calculated from the average volume of iodine used and reported in the **Graph 1, 2, and 3**.



Graph 1: Average Concentration of ascorbic acid in mg/100g of citrus fruits

Graph 2: Average concentration of ascorbic acid (mg/100ml) of citrus fruit juice.

Discussion of results:

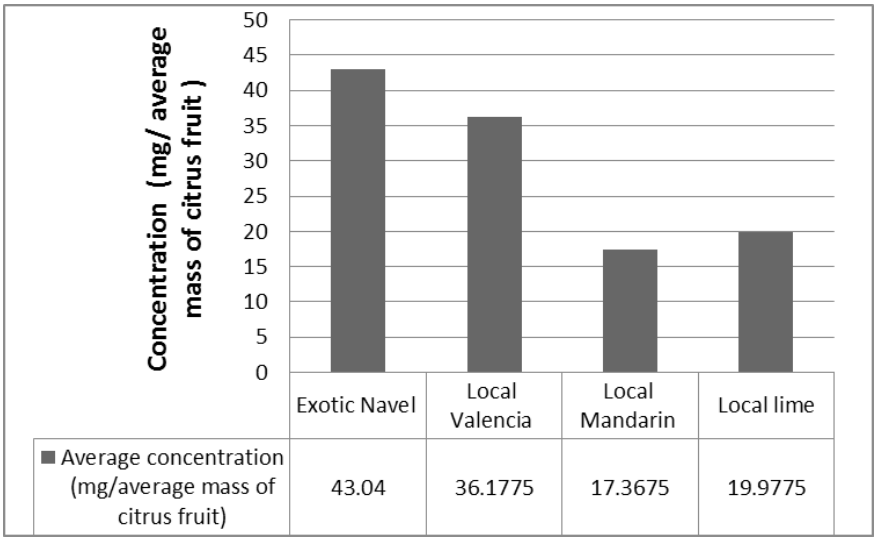
Amount of ascorbic acid in the varieties of the citrus fruits

The first objective of the study was to determine the amount of ascorbic acid in the exotic novel orange and the local varieties of citrus fruits. The **graph 3** compares the average concentration of ascorbic of average mass of citric fruits. From the graph, the average concentration of ascorbic acid of Exotic Noval orange, Local Valencia orange, Local Mandarin and Local Lime lemon, in mg/average mass of citrus fruit, are 43.04, 36.18, 17.37 and 19.98 respectively. So Exotic Novel orange has the highest amount of ascorbic acid per average mass of the citrus fruits. **Graph 1** compares the average concentration of ascorbic acid of the varieties citrus fruits in mg/100g. Exotic Novel orange has the highest ascorbic acid concentration of 23.37mg/100g of the fruit, followed by Local Valencia orange, Local Mandarin and Local Lime lemon with the concentration 21.63mg/100g, 20.04mg/100g and 12.01mg/100g respectively. Therefore Exotic Novel orange has the highest amount of ascorbic per 100g of the fruit and the Local lime has the lowest. The P – value of the mean concentrations of the ascorbic acid was found to be 0.003. This indicated that there is a significant difference in ascorbic concentration in the different citrus fruit varieties. In terms of mg/100ml of juice, Exotic Novel orange is found to have the highest value of ascorbic as shown in **graph 2.** The difference of amount of ascorbic acid in this study is due to species and variety of the citrus fruits. For example Novel orange and Valencia orange have the same species but different variety hence have different amount of ascorbic acid in 100g of the fruit. Orange and lime have different species hence have different concentration of ascorbic acid. Among the citrus fruit variety Navel orange is the best too be consumed for the prevention of non – communicable diseases because it has highest ascorbic acid as compare to the other varieties in the study.

The results of this current study were compared with other published literatures. In this study it was found that Exotic Navel orange contained 43.04 milligrams of ascorbic acid per average mass of a citrus fruit. The expected value for a standard medium orange is 60mg - 70 mg ascorbic acid in 1 fruit (Levine *et al*., *1999*). In another study carried out by Haan (2015) the experimentally determined value of ascorbic acid in 1 piece of orange was 42.53 (±0.80) milligrams of ascorbic acid. This is closer to the value of the current study. In the study of Izuagie and Izuagie (2007) the average ascorbic acid in orange was 60.00mg/100ml, in Tangerine/Mandarin was 44.40mg/100ml and in Lime was 30.60mg/100ml juice. In the current investigated results, the average ascorbic acid was found to be highest in Exotic Novel orange (65.50mg/100ml) followed by Local Valencia (62.43mg/100ml, Local Mandarin (52.38mg/100ml) and Local Kaffir Lime (41.70mg/100ml). Izuagie and Izuagie found the ascorbic acid of orange by Iodometric titration was lower than this current finding from the same method. This difference may be the reason that ascorbic acid content of citrus fruits is never stable but varies with some factors which include position on the tree; climatic/ environmental conditions; ripening stage; species and variety of the citrus fruits, regional varieties of fruits as well as temperature (Ghani *et al*., 2016). Different techniques of measuring and squeezing process may also affect the ascorbic acid content of fruit juices. Factors including amount of nitrogen fertilizers used in growing the plant and various physical conditions such as light can also affect the concentration of ascorbic acid in fruits. The amount of ascorbic acid content in fruit juices can also be affected by the type and duration of storage (Nishanta *et al*., 2016).

Quantity of citrus fruit needed to provide RDA of ascorbic acid.

The second objective of the study was to determine the quantity of citrus fruit needed for a person to eat per day to provide a Recommended Daily Allowance (RDA) of ascorbic acid for healthy living. The average amount of ascorbic acid present in the average mass of citrus fruit has been shown in **Graph 3**.



Graph 3: Average Concentration of Ascorbic Acid in mg per Average Mass of Citrus Fruits.

From this graph, we can deduce the number and size of fruits that should be taken to balance the daily need of ascorbic acid in the human body. It was calculated that 183.98g of Novel orange produced 43.04mg of ascorbic acid, so 192.55g of Novel orange (one orange) can yield 45.00mg of ascorbic acid. Therefore to meet the 45mg recommended daily allowance (RDA) of ascorbic acid for adult, one medium size orange of mass 192.55g should be taken daily by an adult. Taking two and half pieces of Local Mandarin would supply 45.00 mg of ascorbic acid required for daily need. And consuming one and a quarter of Local Valencia every day is enough for an adult to get recommended amount of ascorbic acid daily. However, about two and a quarter Local Kaffir lime will have to be eaten to meet the same daily requirement of vitamin C for an adult person. Quantity of citrus fruits needed to meet a Recommended Daily Allowance of ascorbic acid per person were calculated and presented in **Table 1** below:

Table 1: Quantity of Citrus Fruit Needed to Provide RDA of Ascorbic acid

Citrus fruits	Group/age	Recommended Vit.C (mg/day)	Number of citrus fruit/day	Mass of citrus fruit(g) per day	Juice volume (ml) per day.
Exotic Navel orange	0 –6 months	25.00	0.50	106.00	38.00
	7 months -6yrs	30.00	0.75	128.00	45.00
	10 -18 years.	40.00	1.00	171.00	61.00
	19 years above	45.00	1.00	193.00	68.00
	Pregnant women	55.00	1.25	235.00	83.00
	Lactating women	70.00	2.00	300.00	106.00
Local Valencia orange	0 –6 months	25.00	0.75	116.00	40.00
	7 months -6yrs	30.00	1.00	139.00	48.00
	10 -18 years.	40.00	1.00	186.00	65.00
	19 years above	45.00	1.25	209.00	73.00
	Pregnant women	55.00	1.50	255.00	89.00
	Lactating women	70.00	2.00	325.00	113.00
Local Mandarin	0 –6 months	25.00	1.50	101.00	48.00
	7 months -6yrs	30.00	1.75	122.00	58.00
	10 -18 years.	40.00	2.25	162.00	77.00
	19 years above	45.00	2.50	183.00	87.00
	Pregnant women	55.00	3.25	223.00	106.00
	Lactating women	70.00	4.00	284.00	135.00
Local kaffir lime	0 –6 months	25.00	1.25	208.00	60.00
	7 months -6yrs	30.00	1.50	249.00	72.00
	10 -18 years.	40.00	2.00	332.00	96.00
	19 years above	45.00	2.25	374.00	108.00
	Pregnant women	55.00	2.75	457.00	132.00
	Lactating women	70.0	3.50	581.00	168.00

4. CONCLUSION

The concentration of ascorbic acid varies from one citrus fruit variety to another. Exotic Navel orange, Local Valencia orange, Local Mandarin and Local Kaffir Lime have concentrations of 23.37mg/100g, 21.63mg/100g, 20.04mg/100g and

12.01mg/100g respectively. The P – value of 0.003 indicated that there is a significant difference in the mean concentrations of the ascorbic acid in the different citrus fruit varieties. The highest concentration of ascorbic acid was registered in Exotic Navel orange and the lowest in Local Kaffir lime. The Iodometric titration performed on a whole Exotic Navel orange fruit showed that a whole fruit contained 43.04 milligrams of ascorbic acid per 1 fruit which was near to 42.53 mg/1 fruit (Haan,2015), but far from the expected value (60 – 70 mg/ 1 fruit). This difference may be the reason that ascorbic acid content of citrus fruits varies with some factors which include position on the tree; climatic/environmental conditions which vary from one place to another. It was determined that consuming one and a quarter of Local Valencia every day is enough for an adult to get recommended amount of ascorbic acid daily. Exotic Navel orange, Local Valencia Orange and Local Mandarin provide high amount of ascorbic acid per 100g of the fruits, so they are recommended for consumption to provide recommended daily allowance of ascorbic as compare to sour Local Kaffir Lime with low concentration of ascorbic acid.

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APPENDICES-A

LIST OF APPENDICES

Appendix 1: Photographs of the varieties of citrus fruits used in the study.

Seedless Exotic Navel oranges (*Citrus sinensis*),

Local Kaffir Lime (*Citrus hystrix*)

Local Mandarin Oranges (*Citrus reticulate)*

Local Valencia (*Citrus sinensis)*

Appendix 2: Mass of Fresh Fruits and Volume of the Juices (before dilution).

Varieties of citrus fruits	**Sample 1**		**Sample 2**		**Sample 3**		**Sample 4**	
	Mass (g)	Volume (m/l)	Mass (g)	Volume (m/l)	Mass (g)	Volume (m/l)	Mass (g)	Volume (ml)
Exotic Navel oranges (*Citrus sinensis*).	187.09	68.1	185.06	66.2	182.65	64.9	181.14	60.8
Local Valencia (*Citrus sinensis).*	175.2	59.1	176.1	62.34	143.2	49.37	174.2	61
Local Mandarin Oranges (Citrus reticulate).	108.32	42.3	90.64	35.1	77.11	28.4	70.1	28.1
Local Kaffir Lime (Citrus hystrix)	158.77	45.2	157.73	45.4	192.91	57.6	156.53	44.7

Appendix 3: Comparing Ascorbic Acid Concentration in Citrus Fruits.

				Concentration of Ascorbic Acid				
Sample	**Citrus fruits varieties**	**Weight (g)**	**Juice volume (ml)**	**mg/1 citrus fruit.**	**mg/g**	**mg/100g**	**mg/ml**	**mg/100ml**
1	Exotic Navel	187.090	68.100	44.660	0.238	23.800	0.655	65.500
	Local Valencia	175.200	59.100	36.900	0.211	21.060	0.6243	62.430
	Local Mandarin	108.320	42.300	22.160	0.205	20.450	0.5238	52.380
	Local lime	158.770	45.200	18.850	0.119	11.870	0.417	41.700
2	Exotic Navel	185.060	66.200	43.670	0.236	23.600	0.660	65.960
	Local Valencia	176.100	62.340	39.000	0.221	22.140	0.626	62.560
	Local Mandarin	90.640	35.100	18.190	0.201	20.060	0.518	51.820
	Local lime	157.730	45.400	18.190	0.118	11.760	0.410	41.040
3	Exotic Navel	182.650	64.900	43.470	0.238	23.790	0.670	66.970
	Local Valencia	143.200	49.370	30.770	0.215	21.480	0.623	62.320
	Local Mandarin	77.110	28.400	14.560	0.189	18.880	0.513	51.300
	Local lime	192.910	57.600	24.480	0.127	12.680	0.425	42.500
4	Exotic Navel	181.140	60.800	40.360	0.223	22.28	0.664	66.400
	Local Valencia	174.200	61.000	38.040	0.218	21.830	0.623	62.340
	Local Mandarin	70.100	28.100	14.560	0.208	20.770	0.518	51.810
	Local lime	156.530	44.700	18.390	0.118	11.750	0.411	41.140

Appendix 4: Average Result of Ascorbic Acid Concentration in the Citrus Fruits**.**

			Concentration				
Citrus fruits	**Mass (g)**	**volume (ml)**	**mg/average mass**	**mg/g**	**mg/100g**	**mg/ml**	**mg/100ml**
Exotic Navel	183.985	65.000	43.040	0.234	23.368	0.662	66.208
Local Valencia	167.175	57.9525	36.178	0.216	21.628	0.624	62.413
Local Mandarin	69.0175	33.475	17.368	0.201	20.040	0.518	51.828
Local lime	166.485	48.225	19.978	0.120	12.015	0.416	41.595

Appendix 5: One-way Completely Randomized ANOVA of average concentration of Ascorbic acid (mg/100g)

Samples	**Exotic Navel**	**Local Valencia**	**Local Mandarin**	**Local Lime**
1	23.800	21.060	20.450	11.870
2	23.600	22.140	20.060	11.760
3	23.790	21.480	18.880	12.680
4	22.280	21.830	20.770	11.750
n	4	4	4	4
X	23.367	21.628	20.040	12.015
s	0.731	0.465	0.826	0.447
$\mathbf{X}_{ave}$	19.262			

source	**df**	**SS**	**MS**	**F**	**P-value**
treatments	3.000	302.300	100.767	247.020	0.0027
error	12.000	4.895	0.408		
total	15.000	307.195			