American Scientific Research Journal for Engineering, Technology, and Sciences (ASRJETS)
ISSN (Print) 2313-4410, ISSN (Online) 2313-4402

http://asrjetsjournal.org/

Quantification of Lycopene from Tomatoes and Watermelons by Using Beer-Lambert Principle

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Abstract

Red tomatoes and red-fleshed watermelons contain a high level of lycopene. It is well known that lycopene is precursor to vitamin A. In Uganda, and East Africa in general, there are so many tomato and watermelon varieties with little information on their lycopene content. However, no study has been done to estimate the quantity of lycopene in fresh tomatoes and watermelons in Uganda. The objective of this study was to quantify lycopene by using Beer-Lambert Principle. The varieties were bought from Nakulabye market in Kampala**,** and extracted by using solvent system of acetone/ethanol/ hexane (5ml/5ml/10ml). Stirring on ice was done for 15 minutes. 3 ml of deionized water was added after shaking. Samples were shaken for 5 minutes on ice and then left at room temperature for 5 minutes to allow the separation of phases. The lycopene layer were then separated and scanned in UV-VIS spectrophotometer. The results showed that the lycopene concentration ranged from 27 µg/g to 115 µg/g with % relative error ranged from 2.86 to 3.14, the standard error ranged from ± 0.013 to ± 0.015, and standard deviation ranged from 7.38 to 29.5. Both watermelons and tomato varieties contain the appreciable quantities of lycopene which is the significant nutrient for human body in daily life.

Keywords: Lycopene; concentration; fortified; vitamin A; spectrophotometry; solvent system; deionized.

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1. Introduction

Lycopene is a pigment which is principally responsible for the characteristic deep-red colour of ripe tomato and other fruits [1]. It occurs naturally as carotenoid in tomato, watermelon and papaya [2]. It is a C_{40}-carotenoid made up of eight isoprene units. It is insoluble in water but only soluble in oils and in organic solvents such as benzene, chloroform, carbon disulphide, ether, petroleum ether and hexane. It is almost insoluble in methanol, cyclohexane and ethanol [2]. Because of its non-polarity, lycopene in food preparations will stain any sufficiently porous material, including most plastics. While a tomato stain can be fairly easily removed from fabric (provided the stain is fresh), lycopene diffuses into plastic, making it impossible to remove with hot water or detergent [2]. Dietary lycopene has ability to reduce the risk of chronic diseases such as cancer and coronary heart disease [3]. In human health lycopene plays the role of an antioxidant and has benefits to other mechanisms including intercellular gap junction communication, hormonal and immune system modulation and metabolic pathways [3]. Its presence in diet makes considerable interest as it exhibits a physical quenching rate constant with singlet oxygen, almost twice as that of β-carotene [1]. Lycopene is precursor to vitamin A and there is deficiency of vitamin A in the normal Uganda diet, hence a lot of foodstuffs are fortified with vitamin A [5]. Solubility of lycopene in different solvents or solvents systems show that lycopene differ in degree of solubility from one solvent to another and hence it is not ease to extract the whole amount of lycopene from tomato or watermelon. Lycopene is fat-**soluble**, so the vegetable cooking oil is said to help absorption of lycopene in human body since cooking the tomato in a little fat, such as olive oil, breaks down the cell walls and makes the fat-soluble lycopene more available [4]**.** Lycopene is non-toxic and is commonly found in the diet, but cases of excessive carotenoid intake have been reported. In a middle aged woman who had prolonged and excessive consumption of tomato juice, her skin and liver were colored orange-yellow and had elevated levels of lycopene in her blood. After three weeks on a lycopene-free diet her skin color returned to normal [2]. Generally, water facilitates absorption of food in human body Eating fruits with empty stomach facilitates absorption of fruits nutrients including lycopene. Lycopene can be absorbed more efficiently by the body after it has been processed into juice, sauce, paste, or ketchup [1]. Juice is more preferable since the degradation of lycopene is minimized because it can be done at low or normal temperature [6]. However, there is little information concerning with estimated quantity of lycopene in these fruits. The objective of this study was to quantify the extractable lycopene available in watermelon and tomato varieties by using Beer-Lambert Principle, in which both theoretical and experimental methods were used. This study was significantly giving the estimate of lycopene possibly to be obtained from fruits and the standard deviation and error obtained during measurement. The limitations of this study were that the extraction of lycopene layer together with the analysis of lycopene should be done in slightly dark room of laboratory, the reagents used should be analytical grade, lycopene solution scanned in UV-VIS should be very dilute and the sampled fruits should be arrived the reasonable ripen point.

2. Materials and Methods

2.1 Study area

This study was conducted at Nakulabye market for sampling area and the laboratory work was done in Makerere

University, Kampala, Uganda.

2.1 Apparatus

Beakers, conical flasks, measuring cylinders, droppers, analytical balance, aluminium foil for wrapping container containing the prepared solution of standard lycopene solution, and UV-1700 CE Spectrophotometer (Shimodzu, Kyoto, Japan).

2.2 Chemicals

Lycopene Standard (0.04mg/ml), deionized water, acetone, hexane, ethanol, Ethyl acetate and pet-ether.

2.3 Fruits

2.3.1 Tomatoes

Elliptical tomato (*Lycopersicon esculentum*), small spherical tomato (*Solanum quitoense*) and large spherical tomato (*Solanum lycopersicum cerasiforme*) varieties.

2.3.2 Watermelon (Citrullus lanatus)

Red fleshed watermelon

2.4 Sampling

Samples of tomato and watermelon were bought from Nakulabye market in Kampala.

2.5 Sample processing

Samples of tomato and red-fleshed watermelon were cut separately into smallest possible pieces and then ground to most possible small particles using mortar and pestle.

2.6 Sample extractions

Grounded mass of samples between 0.3 g and 0.6 g were measured. Five ml of acetone, 5 ml of ethanol and 10 ml of hexane were added to each sample. Stirring on ice was done for 15 minutes. Three ml of deionized water was added after shaking. Mixtures of samples and added solvents were shaken for 5 minutes on ice and then left at room temperature for 5 minutes to allow the separation of both phases. Lycopene layer was then analyzed by scanning in UV- VIS Spectrophotometer and the concentration of lycopene was calculated by using Beer-Lambert equation. The absorbance of lycopene layer (upper layer) was measured in a 1-cm-parth length quartz cuvette at 503 nm blanked with hexane after suitable calibration of instrument with hexane [7].

2.7 UV-VIS Spectrophotometer scans

The optimum range from 800 nm in the visible region to 200 nm in the ultraviolet was chosen. The instrument sensitivity was adjusted so that the strongest peak reached 75 – 100% of the vertical scale. A cuvette filled with hexane was allowed to run as the blank (baseline). The cuvette was then filled three-quarter full of lycopene layer and run on a UV-VIS spectrum [8].

2.8 The formulae used in calculations of data

$$A = \varepsilon l c$$

The content of lycopene in samples was estimated by two methods: theoretical method and experimental method.

2.8.1 A theoretical method

A method in which the molar extinction coefficient of lycopene in hexane (17.2×10^4 $M^{-1}cm^{-1}$) was used in Lambert-Beer law which was described as:

$(A_{503}) = \varepsilon bc$

Where (A_{503}) = Absorbance at 503 nm

ε = constant measured in litre $mol^{-1}cm^{-1}$

b = length of cuvette in cm

c = lycopene concentration in $mollitre^{-1}$ [7].

By properly substituting the molar extinction coefficient of lycopene in hexane (17.2×10^4 $M^{-1}cm^{-1}$), molecular weight (536.9) and changing the unit, the final equation will be

Lycopene content (mg/Kg) = A_{503} x 31.2/g tissue [7].

2.8.2 Experimental data based method

A method obtained from calibration curve. In this case, from graph of calibration curve and by appropriate substitution in the Lambert-Beer law equation, it can be found that

Lycopene content (mg/kg) = $(A_{503} - 0.0007)$ x 30.3/g tissue [7]

These two formulae can also used to compare the data with those of the literature

Relative error (%) = (a-b)/a [7]

where

a = lycopene content (μg/g) using theoretical data (fresh weight)

Theoretical data means the expected data would be obtained if the conditions are totally achieved [7].

The reason for calculating the theoretical data was to get the data of mass of lycopene if absorbance of light would occur in expected conditions that obey the Beer-Lambert Principle. These data help to calculate the error and percentage error in measurement of mass of lycopene from actual absorbance of light by using the Beer-Lambert equation.

b = Lycopene content (μg/g) using experimental data (fresh weight).

In the general case when the results of measurements expressed as

$X_n \pm S_n$, $X_m \pm S_m$, $X_l \pm S_l$,……..(X and S are arithmetical mean and standard deviation respectively) are to be combined.

The weights that give the least overall error are proportional to $1/S_n^2$, $1/S_m^2$, $1/S_l^2$,

A weight mean defined as:

$$X_{n,m,l} = [1/ (S_n^{-2} + S_m^{-2} + S_l^{-2} + ……..)][(X_n/ S_n^2 + X_m/ S_m^2 + X_l/ S_l^2 + …….)]$$

is regarded as the best combined estimate of the true value.

The standard error of the weight mean is given by

$$S^{-2}_{n,m,l} = S_n^{-2} + S_m^{-2} + S_l^{-2} \qquad [9].$$

3. Results and Discussion

3.1 Concentrations of lycopene from watermelon and tomato samples

The absorbance of lycopene standard at wavelength of 503 nm together with their respective concentrations of lycopene scanned on UV-VIS spectrophotometer varied from 0,018 to 0.06 with R^2 = 0.966 (Table 1.0). The absorbance of lycopene at wavelength 503 nm was chosen so as to avoid the interference of other carotenoids in the samples though the absorbance at this wavelength value is not the absorbance at the greatest of lycopene in hexane [7]. In watermelon (*Citrulus lanatus*) concentration of lycopene varied from 69.6μg/g to 97.2 μg/g of sample with average concentration of 79 μg/g and standard deviation of 9.98 with the standard error of the weight mean of ±1.13%. Quantity of lycopene in tomatoes showed minor variations between species with highest average concentration found in *Lycopersicon esculentum*. In *Lycopersicon esculentum* species the level of lycopene varied from 48.5 μg/g to 115 μg/g of sample and its average concentration was 87.4 μg/g. Standard error of the weight mean was ±1.148%.

Number of µg of lycopene per gram of fresh sample in *Solanum lycopersicum cerasiforme* ranged from 53.5 µg/g to 73.7µg/g while that of *Solanum quitoense* was between 27.0 µg/g and 56.0 µg/g of sample. Their average concentrations were 64.9 µg/g and 50.9 µg/g in *Solanum lycopersicum cerasiforme* and *Solanum quitoense*, respectively. Their standard deviations were 7.38 and 14.8 respectively; their standard errors of the weight mean were ±1.32% and ±1.48% respectively.

The experimental measured mean weight of tomatoes varied from 50 µg/g to 87µg/g as recorded as the results of applying Beer-Lambert equation. Comparing with data obtained by theoretical measurement of weight of lycopene, the relative error of the experimental measurement of weight of lycopene varied from 2.86 to 3.14.

The experimental measured mean weight of watermelon was 79 µg/g. The relative error of experimental measurement from theoretical measurement varied between ± 0.028 and ±0.031. The standard relative error of weight mean varied from ± 0.0131 to ± 0.0148 and standard deviation varied from 7.38 to 29.4 (Table 2). Theoretical measurement could be obtained by calculating the expected quantity of lycopene that was expected from the measured sample used for extraction of lycopene that used in Beer- Lambert equation for absorption spectra.

Comparison of the determined lycopene in this study with those reported in the literature, in which the calibration curve of lycopene standard has R^2 = 0.9999, has shown that concentrations of lycopene using experimental data (fresh weight) were 63.1, 46.6 and 52.9 mg/kg for watermelon (with average concentration of 54.2 mg/kg); 89.0, 84.0 and 94.7 mg/kg for tomato (with average concentration of 89.2 mg/kg). Lycopene content using theoretical data were 64.9, 48.0 and 54.4 mg/kg for watermelon; 91.5, 86.4 and 97.5 mg/kg for tomato. The relative error were 2.77, 2.91 and 2.76 for watermelon; 2.73, 2.78 and 2.87% for tomato [7]. The observed variations were attributed by the quality of the fruits. Variety and growing environment are among the causes of the difference [10].

The mean concentration of experimental weight of 79 µg/g of lycopene from watermelon from this study >> the mean concentration of experimental weight of 54.2 µg/g of lycopene from watermelon recorded from literature. Also the mean concentrations of experimental weight of 50.9 µg/g, 64.9 µg/g and 87.4 µg/g of lycopene from tomato from this study < the mean concentration of experimental weight of 89.2 µg/g of lycopene from tomato recorded from literature. Remind that 1 µg/g = 1 mg/kg.

Table 1: Concentrations and their respective absorbance of lycopene standard

	1	2	3	4	5
Concentration (mg/litre)	0.4	0.5	0.8	1.0	2.0
Absorbance (A_{503})	0.020	0.018	0.035	0.035	0.061

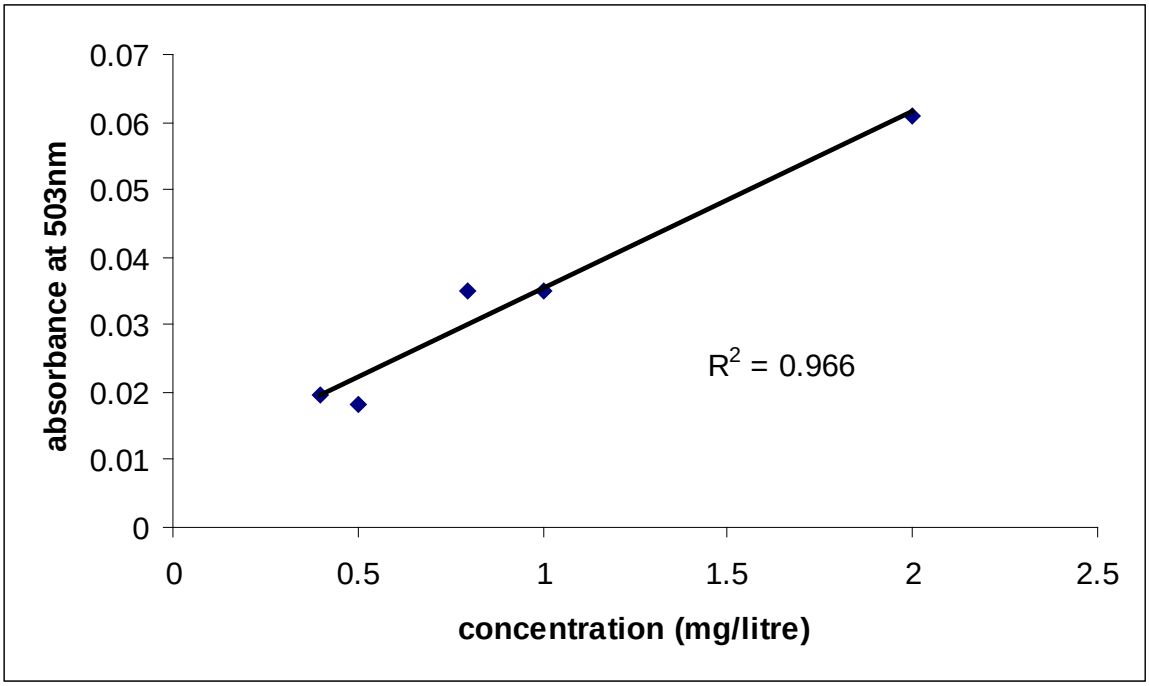

Figure 1: Absorbance versus concentration (mg/litre) in hexane of lycopene Standard

The following Figure 2 shows the comparison of quantity of lycopene obtained from experimental data for both watermelons and tomato species.

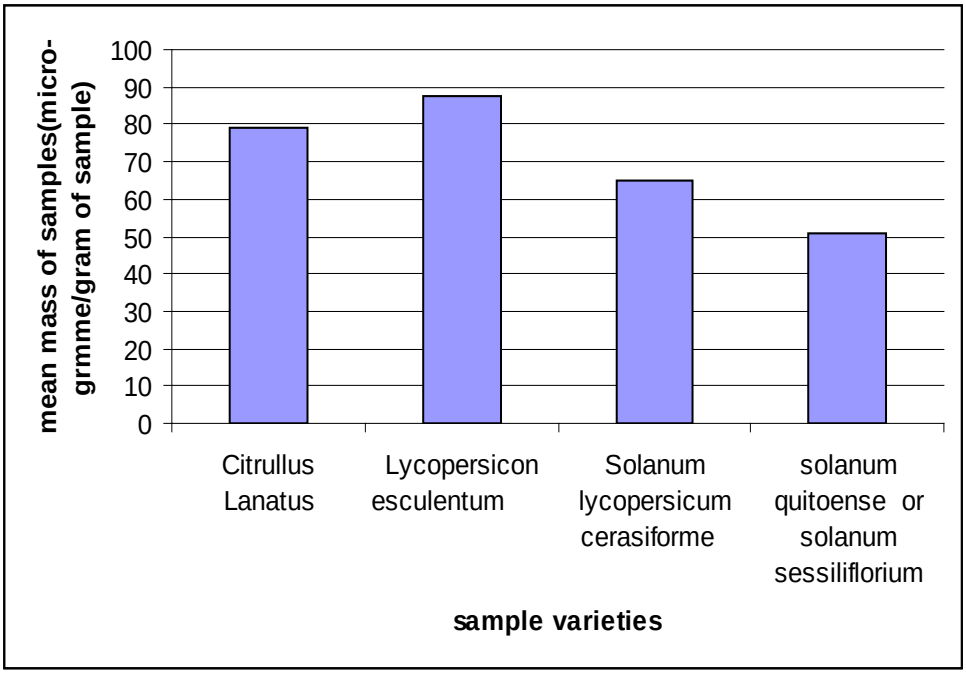

Figure 2: Variation of mean mass of samples calculated from Beer-Lambert equation after extracted from their respective samples and scanned on UV-VIS spectrophotometer

Table 2: Quantities of lycopene from tomato and watermelon samples using both theoretical and calculated data

Samples		S/N of samples	Mass of sample in g	Absorbance (nm)/ A_{503}	Lycopene content(μg/g) using experimental data (fresh weight)	Theoretical data (fresh weight).	Relative error (%)	Mean mass (μg/g) of experimental weight	The standard error of the weight mean (%)	Variance	Std deviation
Citrullus lanatus		1	0.353	0.810	69.6	71.7	2.97	79	±1.31	98.3	9.91
		2	0.417	1.34	97.2	100	2.95				
		3	0.516	1.38	81.2	83.6	2.86				
		4	0.683	1.69	74.9	77.1	2.92				
		5	0.805	1.91	72.0	74.2	2.92				
Lycopersicon esculentum		1	0.321	1.22	115	118	2.94	87.4	±1.48	869	29.5
		2	0.354	0.744	63.5	65.5	2.98				
		3	0.402	0.644	48.5	50.0	2.99				
		4	0.690	1.50	65.7	67.6	2.93				
		5	0.823	1.50	55.1	56.8	2.93				
Solanum lycopersicum cerasiforme		1	0.319	0.722	68.5	70.6	2.98	64.9	±1.32	54.5	7.38
		2	0.412	0.945	69.5	71.7	2.96				
		3	0.496	1.21	73.7	75.9	2.94				
		4	0.584	1.14	59.2	61.0	2.94				
		5	0.736	1.30	53.5	55.2	2.94				
Solanum quitoense		1	0.300	0.269	27.0	27.9	3.14	50.9	±1.48	182	13.5
		2	0.453	0.564	37.7	38.8	3.01				
		3	0.534	0.988	56.0	57.7	2.95				
		4	0.574	0.843	44.5	45.8	2.97				
		5	0.744	1.01	41.2	42.5	2.95				

3.2 Correlation of lycopene of experimental weights from watermelon and tomato varieties

3.2.1 From Citrullus lanatus and Lycopersicon esculentum

The correlation of level of lycopene extracted from *Citrullus lanatus* and *Lycopersicon esculentum* was well shown in following diagram:

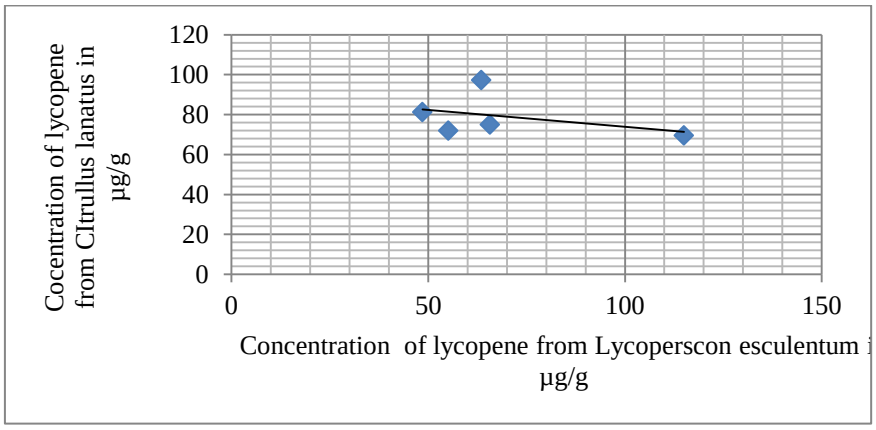

Figure 3: Correlation of lycopene concentrations from *Citrullus lanatus* and *Lycopersicon esculentum*

Correlation = -0.40057

The graph shows that the concentration of lycopene extracted from watermelon (*Citrullus lanatus*) increased with the decrease concentration of *Lycopersicon esculentum*. Since the variation is negatively increased it seemed to what extent these fruits differ in their lycopene content. Watermelon contains higher quantity of water content than *Lycopersicon esculentum* species of tomato.

3.2.2 From Citrullus lanatus and Solanum lycopersicum cerasiforme

The correlation of levels of lycopene extracted from *Citrullus lanatus* and *Solanum lycopersicum cerasiforme* is well shown in following Figure 4:

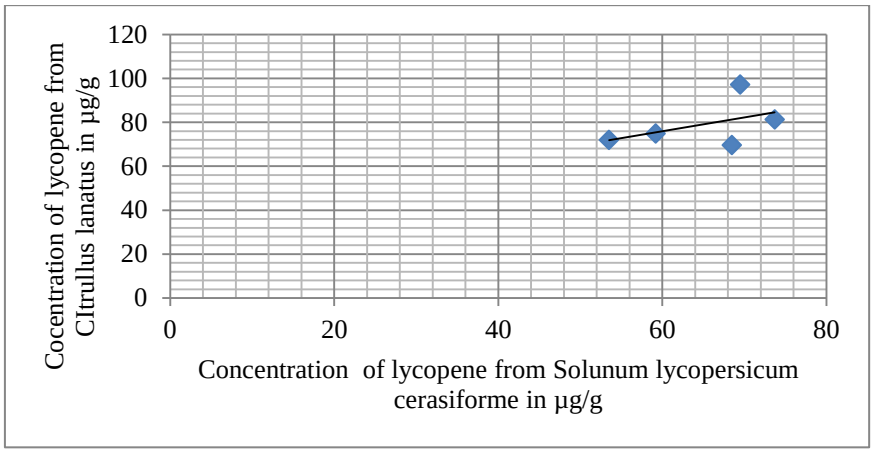

Figure 4: Correlation of lycopene concentrations from *Citrullus lanatus* and *Solanum lycopersicum cerasiforme*

Correlation = 0.470

The correlation in the above graph shows that the concentration of lycopene extracted from watermelon (*Citrullus lanatus*) increased with the increase concentration **of** *Solanum lycopersicum cerasiforme*. Since the variation is positively increased it seemed to what extent living organisms can benefit positively to these fruits due to their nutrient (lycopene) contents.

3.2.3 From Citrullus lanatus and Solanum quitoense

The correlation of concentrations of lycopene extracted from watermelon (*Citrullus lanatus*) and *Solanum quitoense* is well indicated in following diagram:

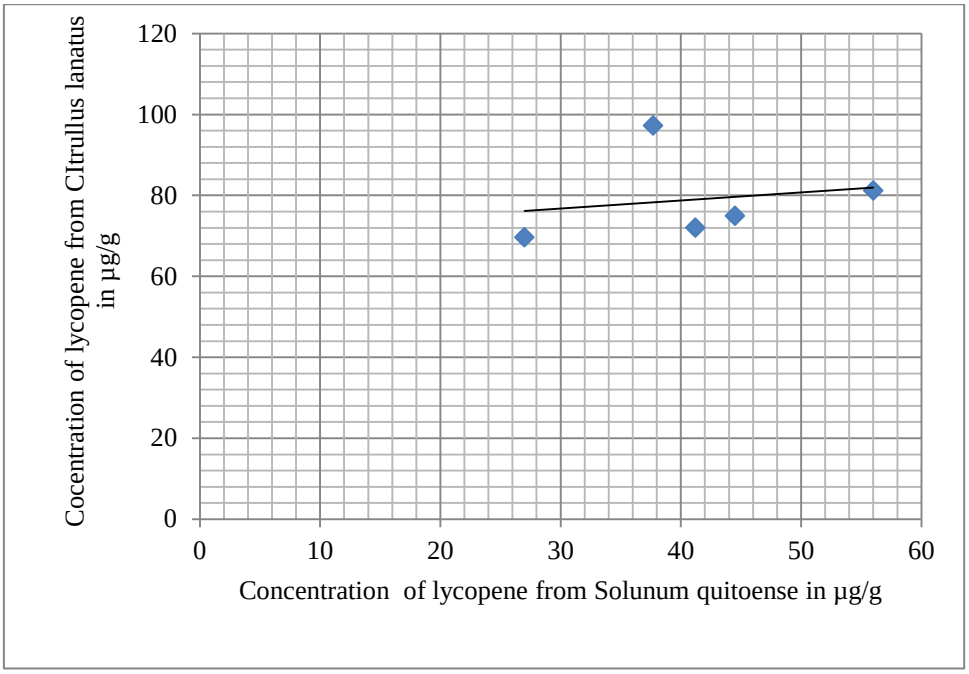

Figure 5: Correlation of lycopene concentrations from *Citrullus lanatus* and *Solanum quitoense*

Correlation = 0.190

The correlation in the above diagram shows that the level of lycopene extracted from watermelon (*Citrullus lanatus*) increased with the increase concentration of *Solanum quitoense*. Since the variation is positively increased it seemed to what extent living organisms can benefit positively to these fruits due to their nutrient (lycopene) contents.

3.3 Correlation of lycopene concentrations within tomato varieties

3.3.1 Between Lycoperscon esculentum **and** *Solanum lycopersicum cerasiforme*

The relationship between levels of lycopene extracted from *Lycoperscon esculentum* and *Solanum lycopersicum cerasiforme* is shown in following Figure 6:

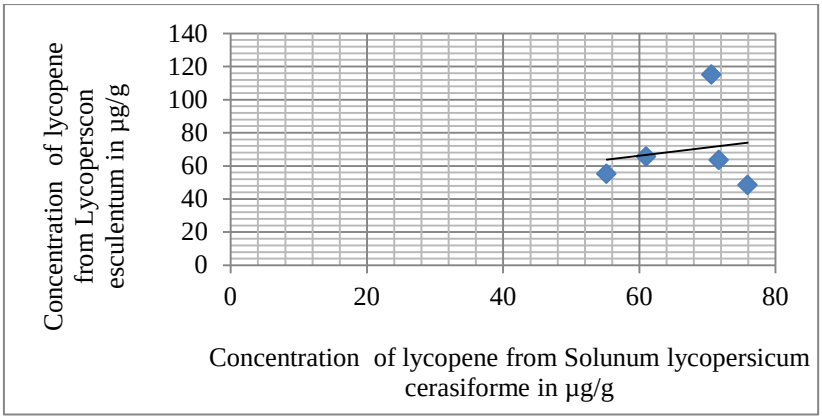

Figure 6: Correlation of lycopene concentration from *Lycoperscon esculentum* and *Solanum lycopersicum cerasiforme*

Correlation = 0.158

The correlation in the above graph shows that the concentration of lycopene extracted from *Lycoperscon esculentum* increased with the increase concentration of *Solanum lycopersicum cerasiforme*. Since the variation is positively increased it seemed to what extent living organisms can benefit positively to these fruits due nutrient (lycopene) contents. It also seems to what extent the varieties of tomato are nearly in similarity.

3.3.2 Between Lycoperscon esculentum and Solanum quitoense

The relationship between levels of lycopene extracted from *Lycoperscon esculentum* and *Solanum quitoense* is shown in following Figure 7:

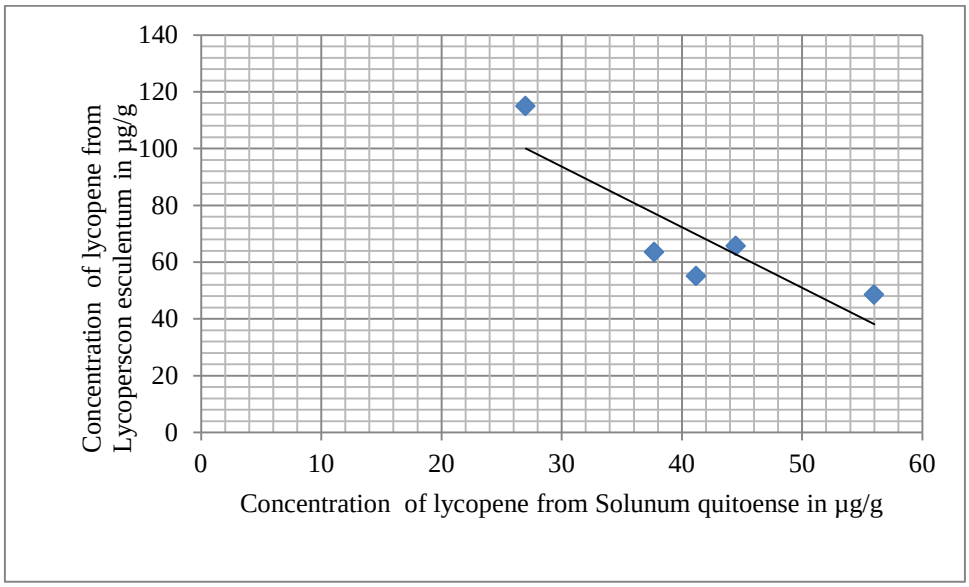

Figure 7: Correlation of lycopene concentrations from *Lycoperscon esculentum* and *Solanum quitoense*

Correlation = -0.856

The graph shows that the concentration of lycopene extracted from *Lycoperscon esculentum* increased with the decrease concentration of *Solanum quitoense*. Since the variation is negatively increased it seemed to what extent these fruits differ in their lycopene content. *Solanum quitoense* contains higher quantity of water content than *Lycopersicon esculentum* species of tomato.

3.3.3 Between Solanum lycopersicum cerasiforme and Solanum quitoense

The following Figure 8 shows relationship between levels of lycopene extracted from *Solanum lycopersicum cerasiforme* and *Solanum quitoense* as shown below:

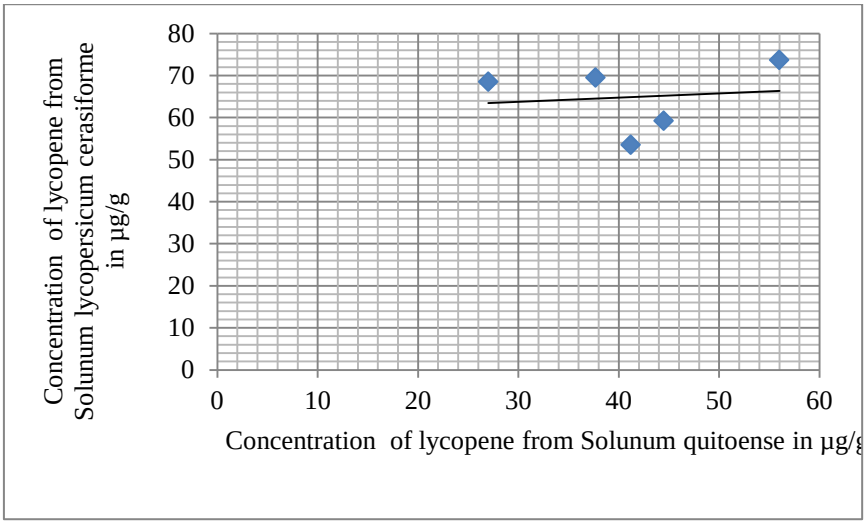

Figure 8: Correlation of concentrations of lycopene extracted from *Solanum lycopersicum cerasiforme* and *Solanum quitoense*

Correlation = 0.127

The correlation in the above graph shows that the concentration of lycopene extracted from *Solanum lycopersicum cerasiforme* increased slightly with the increase concentration of lycopene from *Solanum quitoense*. Since the variation is positively increased it seemed to what extent living organisms can benefit positively to these fruits due to their nutrient (lycopene) contents.

4. Conclusion

The results show that the quantity of extracted lycopene from both tomatoes and watermelons varied. The variation caused by different in conditions, varieties and seasons due to irradiate light [11]. Both watermelons and tomato varieties contain the appreciable quantity of lycopene necessary for daily intake as nutrients in human nutrition.

5. Recommendation

It is far better and advisable to study, investigate and practice the best conditions for growing tomatoes and

watermelons so as to contain the highest possible quantity of lycopene. It is advisable to drink tomato and watermelon juice since the processed lycopene allows the optimum absorption in human body. Extraction of lycopene should also be done when tomato is cooked with cooking oil at very low temperature, such as olive oil, and also by using different solvents. The method used in this study is recommended for practical analytical chemistry classes since it is relatively fast and requires low quantities of organic solvents.

Acknowledgement

I would like to thank madam Jane Namukobe (Makerere University), Mr Yiga Solomon (Makerere University), Dr. Abdalla Ibrahim (SUZA lecturer), Assoc. Prof. Dr. Mohd Ali Sheikh (SUZA lecturer) and Department of Natural Science (SUZA) for their great deal of help.

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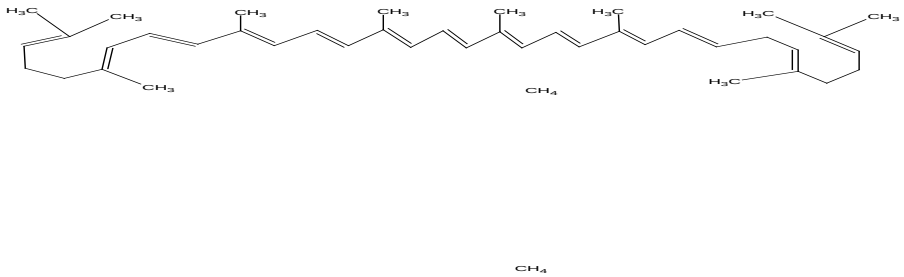
6. Appendix

6.1 Chemical formula of lycopene

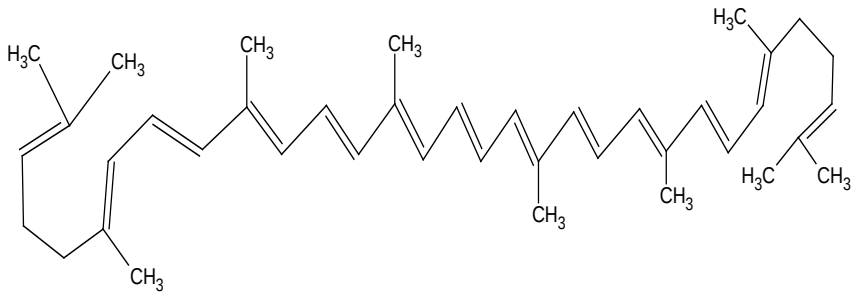
$C_{40}H_{56}$

6.2 Chemical structures of lycopene

cis-lycopene (1) and *all trans*-lycopene (2)



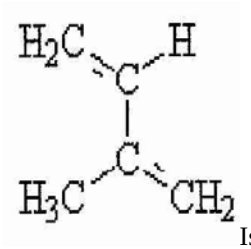
(1)



(2)

Figure 9

6.3 Chemical structure of isoprene unit



Isoprene unit

Figure 10

The isoprene unit is named as 2-methyl 1, 3 butadiene