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Journal of the Kenya Chemical Society 11-1 (2018), 3-9

JOURNAL OF THE KENYA CHEMICAL SOCIETY

Quantification of *all trans*-lycopene, *cis*-lycopene and β-carotene from tomato varieties found in Zanzibar

Haji Khamis[1*], Kituyi Lusweti[2], Haji Mwevura[3]

[1,2]*Department of Chemistry and Biochemistry, P.0.Box 1125-30100, University of Eldoret, Chepkoilel, Kenya*

[3]The State University of Zanzibar (SUZA), P. O. Box 146, Zanzibar, Tanzania

Corresponding author: Haji Khamis Email : khamish03@yahoo.com

Abstract

Tomatoes contain lycopene and β-carotene which have important health benefits. Different varieties are available in Zanzibar Islands and used in daily meal. However, there is little information in Zanzibar about quantity of *all trans*-lycopene, *cis*-lycopene and β-carotene available in those tomato varieties. The objective of this study was to quantify *all trans*-lycopene, *cis*-lycopene and β-carotene from tomato varieties. Samples were collected and extracted using hexane/acetone/ethyl acetate (4:2:1 v/v/v). Extracts were filtered and lycopene layer separated, washed, dried, dissolved in hexane and fractionated by silica gel column. Fractions were collected, dried by blowing with a stream of nitrogen gas and measured their masses by electronic balance. Results showed that mass (in µg/g) of a*ll trans*-lycopene varied from 316 to 827 and from 136 to 931, *cis*-lycopene from 60 to 528 and from 35 to 845, β-carotene from 41 to 405 and from 44.1 to 573, from *Lycopersicon esculentum* and *Solanum quitoense* respectively.Tomatoes contain valuable quantities of lycopene and β-carotene necessary for human nutrients. Best growing conditions which facilitate highest possible quantity of lycopeneand β-carotene should be investigated and used during tomato cultivation.

Keywords: lycopene, quantify, β-carotene, *Lycopersicon esculentum, Solanum quitoense*

1. Introduction

Lycopene is a pigment which is principally responsible for the characteristic deep-red colour of ripe tomato fruits and tomato products (1).It occurs naturally in tomato as a carotenoid and is a major component found in serum component of the fruit (2).It also occurs naturally in certain fruits, vegetables, algae and fungi. Other significant sources are watermelon, pink grapefruit, pink guava, papaya and apricots (3). Lycopene is a C_{40}-carotenoid made up of eight isoprene units. β-carotene, the yellow pigment of the carrot, is the isomer of lycopene. Dietary lycopene has ability to reduce the risk of chronic diseases such as cancer and coronary heart disease (2). In human health lycopene is thought to play the role of an antioxidant and has beneficial properties to other mechanisms including intercellular gap junction communication, hormonal and immune system modulation and metabolic pathways (2).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 scavenges reactive oxygen species, which are aggressive chemicals always ready to react with cell components, causing oxidative damage and loss of proper cell function (4). Lycopene is a precursor of vitamin A (5).Tomatoes (*Solanum lycopersicum)* such as elliptical tomato (*Lycopersicon esculentum*), small spherical tomato (*Solanum quitoense*) and large spherical tomato (*Solanum lycopersicum cerasiforme*) varieties and tomato products are the major sources of lycopene compounds and an important source of carotenoids in the human diet. Cooking the tomato in a little fat, such as olive oil, breaks down the cell walls and makes the fat-soluble lycopene more available (6).

Most of people are not aware of the amount and importance of lycopene and β-carotene available in local available fruits such as tomatoes and red-fleshed watermelons and hence do not give full respect of these fruits. The objective of this study was to separate and quantify all *trans*-lycopene*, cis*-lycopene and β-carotene from selected tomato varieties found in Zanzibar.

2. Experimental (Materials and Methods)

2.1. Apparatus

Beakers, conical flasks, measuring cylinders, droppers, separating funnel, rotary evaporator, glass column chromatography, analytical balance and UV-1700 CE

Spectrophotometer (Shimadzu, Kyoto, Japan), for analysing lycopene samples.

2.2. Chemicals

Silica (chromatography grade), saturated aqueous solution of sodium chloride, 10% potassium carbonate aqueous solution, anhydrous magnesium sulphate, acetone, hexane (analytical grade), ethanol, ethyl acetate, pet-ether, magnesium sulphate, sodium chloride and potassium carbonate. Nitrogen gas was used for drying *all trans*-lycopene, *cis*-lycopene and β-carotene extracts.

2.3. Sample Collection

Samples of tomato were collected from Kilimani/Kidombo farm areas of Zanzibar north district. Only two varieties were available during sampling which was done in summer season and hence sampled. They were elliptical tomato (*Lycopersicon esculentum*) and small spherical tomato (*Solanum quitoense*) varieties.

2.4. Sample Processing

Samples of tomato were cut separately into smallest possible pieces and then ground to most possible small particles using mortar and pestle. A mass 100 g of each variety were measured and extracted.

2.5. Sample Extraction

A sub sample 100 g of grounded tomato was put in a beaker and extracted with a mixture of ethyl acetate, acetone and hexane (1:2:4) and the volume used was 100 ml. This solvent system was used together with using solvent system of acetone/ethanol/ hexane (5ml/5ml/10ml) in quantification of lycopene by using Beer-Lambert principle in which deionized water was added. Lack of deionized water and tendency of ethanol to extract many compounds lead to select the solvent system of hexane, acetone, and ethyl acetate (4: 2:1). The extract was filtered and the filtrate was placed into a second beaker. The extraction of the solid residue was repeated once more with another solvent mixture of the same solvent system and the filtrates were combined together. Two layers (lycopene layer and aqueous layer) appeared in filtrate without water addition. The lycopene layer was separated from filtrate by using separating funnel and concentrated to lowest possible volume by evaporating the solvent under vacuum without heating by using rotary evaporator (7). Before a chromatographic separation, the lycopene-containing organic layer was separated from two layers of original extract without adding some water by using funnel separation, washed by using saturated sodium chloride solution, followed by 10% aqueous potassium carbonate and another portion of saturated sodium chloride solution and then removed any water molecule present with anhydrous magnesium sulphate (8).

2.6. Preparation of Column

A glass column was mounted at a retort stand vertically. A small amount of cotton was placed at the bottom of column followed by hexane (5ml) and clean sand (1cm). Then, activated silica (13 g – 14 g) was mixed with hexane to make thick but pourable slurry and then poured carefully into the column. After settled, slurry of sand and hexane was added at the top of the column with length of about 0.5 cm (7).

2.7. Separation of *all trans*-lycopene, *cis*-lycopene and β-carotene

The concentrated extracts were put to the top of the silica gel column and eluted with hexane. Yellow (β-carotene) band was collected in a small beaker followed by the elution solvent of 15% – 20% (v/v) acetone in hexane was added to the top of the column so as to accelerate the motion of orange-red (*all trans*-lycopene) band and collected into another container. The slow moving yellow-orange (*cis*-lycopene) band was also collected. The extracts were dried by using a stream of nitrogen gas and then weighed.

2.8. UV-VIS spectrophotometer scans

The optimum range from 800 nm in the visible region to 200 nm in the ultraviolet was chosen. A cuvette filled with hexane was allowed to run as the blank (baseline) and then after filled three-quarter full of prepared dilute solution of lycopene in hexane. An ultraviolet–visible spectrum of the lycopene fractions was obtained by using the ocean optics spectrograph of spectrophotometer**.** The instrument sensitivity was adjusted so that the strongest peak reached 75 – 100% of the vertical scale (9).

3. Results and Discussion

3.1. Mass of *all trans*-Lycopene, *cis*-lycopene and β-carotene

Quantity of lycopene in tomatoes showed minor variations between specie with highest average concentration found in *Lycopersicon esculentum.* In *Lycopersicon esculentum* species the level of *all trans*-lycopene varied from 316 µg/g to 827 µg/g of sample and its average concentration of 556±263 µg/g. The amount of β-carotene in this species of tomato varied from 44µg/g to 573µg/g. The average concentration of this carotenoid was 325±192µg/g. Comparison of the determined *all trans*-lycopene, *cis*-lycopene and β-carotene in this study with those reported in the literature show that the study done at Brazil from HPLC of fresh tomato had the results of 1122.2±26.6 µg/g, cis-lycopene was 78.8±13.6, *all-trans*- β-carotene was 52.0±7.4 µg/g and *cis*- β-carotene was 4.7±0.7 µg/g (10)**.** It was also reported that in tomato, the *all trans*-lycopene ranged from 79% to 91% while 9% to 21% was the *cis*-lycopene (11). Usually *cis*-lycopene occurs as trace in mixture of *all trans*–lycopene (12). Literature shows higher

concentration in tomato than those determined in tomato wet weight in this study. Variety and growing environment are among the causes of the difference (13).

Table 1: Mass of *all trans*-lycopene, *cis*-lycopen and ß-carotene extracted from tomato varieties.

Sample used		Mass of sample	Mass (g x 10^{-3})		
		(g)	*All trans*-lycopene	*Cis*-lycopene	β-carotene
Lycopersicon esculentum	1	100	52.0	14.2	6.0
	2	100	70.2	6.0	9.9
	3	100	47.6	52.8	12.7
	4	100	31.6	10.7	40.5
	5	100	64.5	38.7	9.0
	6	100	30.6	33.1	1.1
	7	100	35.2	18.5	11.0
	8	100	57.1	17/1	8.5
	9	100	48.4	13.3	17.7
	10	100	82.7	20.2	12.2
	11	100	62.7	23.3	24.7
	12	100	58.2	27.8	24.9
	13	100	82.0	51.4	33.4
Solanum quitoense	1	100	93.1	84.5	36.3
	2	100	43.6	35.5	30.0
	3	100	38.2	32.0	30.0
	4	100	20.0	20.0	40.0
	5	100	60.0	21.8	49.1
	6	100	40.0	10.0	20.0
	7	100	27.0	17.1	49.3
	8	100	47.0	67.8	17.8
	9	100	49.1	21.8	46.4
	10	100	13.6	41.9	23.0
	11	100	33.2	19.2	41.0
	12	100	57.3	51.8	4.4
	13	100	54.5	57.3	38.2
	14	100	60.0	57.3	57.3
	15	100	21.7	3.5	5.2

Table 2: Concentration of *all trans*-lycopene, *cis*-lycopen and ß-caroten extracted from tomato varieties

Sample used		concentration (µg/g of sample)		
		All trans-lycopene	*Cis*-lycopene	β-carotene
Lycopersicon esculentum	1	520	142	60
	2	702	60	99
	3	476	528	127
	4	316	107	405
	5	645	387	90
	6	306	331	41
	7	352	185	110
	8	571	171	85
	9	484	133	177
	10	827	202	122
	11	627	233	247
	12	582	278	249
	13	820	510	334
Solanum quitoense	1	931	845	363
	2	436	355	300
	3	382	320	300
	4	200	200	400
	5	600	218	491
	6	400	100	200
	7	270	171	493
	8	470	678	178
	9	491	218	464
	10	136	419	230
	11	332	192	410
	12	573	518	44
	13	545	573	382
	14	600	573	573
	15	217	35	52

3.2. Yield of Extract Nutrients

The yields of extracted nutrients depicted the following trend *all trans*-lycopene>*cis*-lycopen>ß-carotene. In both tomato species, *Lycopersicon esculentum* and *Solanum quitoense*, *all trans*-lycopene contributed slightly above half (>50%) of the nutrients and *cis*-lycopen>ß-caroten contributing to the other half.

The following Figure 1 show the % age of *all trans*-lycopene, *cis*-lycopene and β-carotene of *Lycopersicon esculentum*.

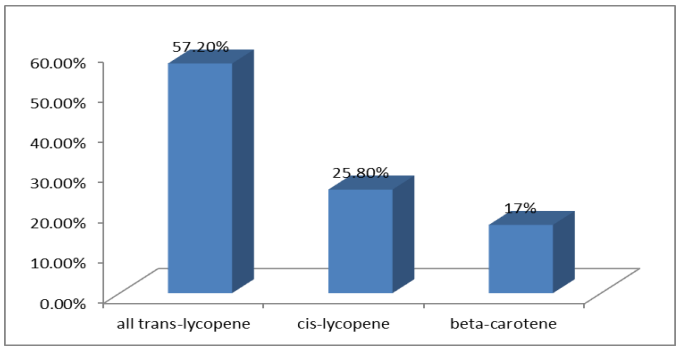

Figure 1: Percentage yield of *all trans*-lycopene, *cis*-lycopene and β-carotene from *Lycopersicon esculentum*

Figure 2 below shows the *Solanum quitoense* nutrients with the % of total food nutrients extracted.

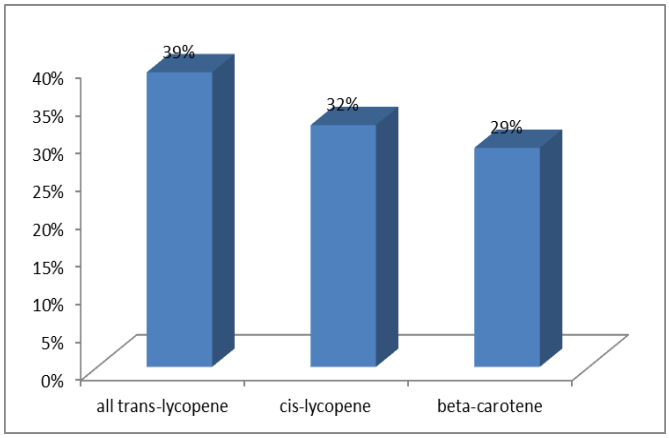

Figure 2: Percentage yield of *all trans*-lycopene, *cis*-lycopene and β-carotene from ***Solanum quitoense***

Figure 3 below shows the finger prints of *all trans*-lycopene and *cis*-lycopene content in tomato.

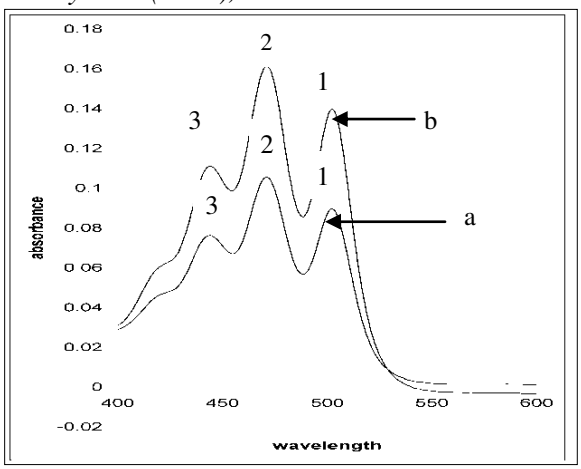

Figure 3: Normal derivative of absorption spectra characteristics of all trans- (b) and cis- lycopene (a) extracted from tomato. Concentration of lycopene scanned in UV-VIS spectrophotometer was 0.01g/litre in hexane solution for each of the two.

In b $\lambda_{1\ max}$ = 502nm, $\lambda_{2\ max}$ = 472nm and $\lambda_{3\ max}$ = 444nm

In a $\lambda_{1\ max\ 1}$= 502nm, $\lambda_{2\ max}$ = 471nm and $\lambda_{3\ max}$ = 444nm

Figure 4 below shows the finger prints of β-carotene content in tomato.

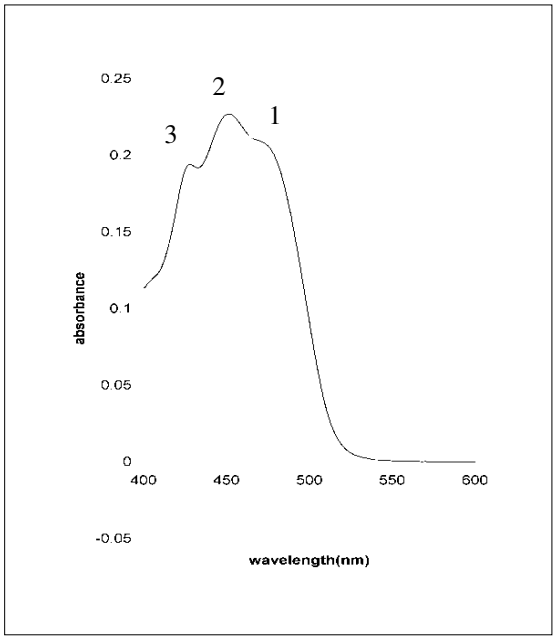

Figure 4: Absorption spectrum characteristics of β-carotene extracted from tomato. β-carotene collected from 1st band from down the separation column. β-carotene was collected and then scanned.

$\lambda_{1\ max}$ = 480nm, $\lambda_{2\ max}$ = 451nm and $\lambda_{3\ max}$ = 428nm

$\lambda_{\ max}$ means wavelength of maximum peak of absorption spectrum.

3.3. Correlations of *trans*-Lycopene, *cis*-lycopene and β-carotene

The correlation between concentrations of *cis*-lycopene and *all trans*-lycopene from *Solanum quitoense*is shown in Figure 5.

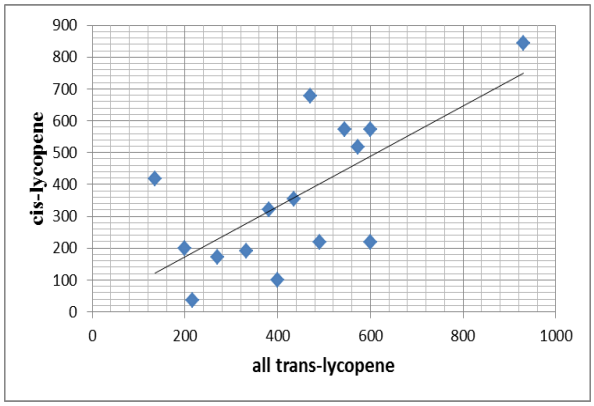

Figure 5: A graph of mass yield of *cis*-lycopene against mass yield of *all trans*-lycopene from *Solanum quitoense*. Correlation (R) = 0.685

The level of *all trans*-lycopene in *Solanum quitoense* increase with the increase concentration of *cis*-lycopene.*All trans*-lycopene is anti-oxidant and precursor to vitamin A hence the increment trend of this carotenoid guarantees the nutrition benefit for intake of this fruit though the biological benefit of *cis*-lycopene in human body has been investigated and well explained.

The correlation of β-carotene and *all trans*-lycopene concentrations from *Solanumquitoense* is shown in Figure 6.

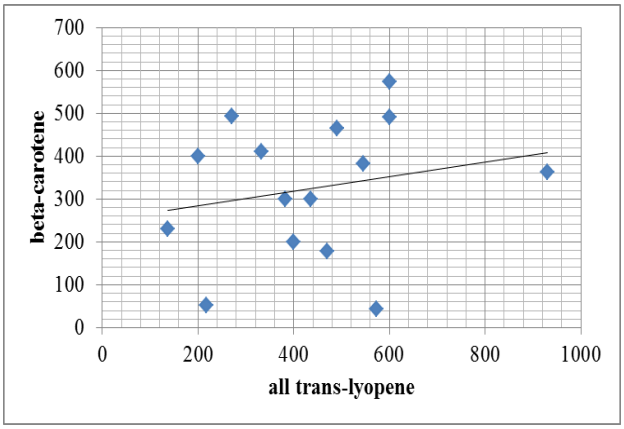

Figure 6: A graph of mass yield of β-carotene against mass yield of *all trans*-lycopene from *Solanum quitoense.* Correlation (R) = 0.215

The graph above shows that the concentration of β-carotene in *Solanum quitoense* increases slightly with the increase of *all trans*-lycopene. Both β-carotene and *all trans*-lycopene are anti-oxidants and precursor to vitamin A hence the slight Increment trend of these two carotenoid guarantee the nutrition benefit for intake of this fruit.

Figure 7 shows the correlation between β-carotene and *cis*-lycopene mass yield from *Solanum quitoense*:

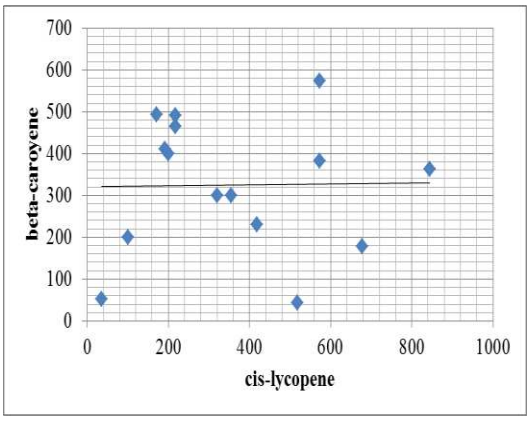

Figure 7: A graph of mass yield of β-carotene against mass yield of *cis*-lycopene from *Solanum quitoense*. Correlation = 0.0147

There is almost nil increase of concentration of β-carotene of *Solanum quitoense* with the increase of *cis*-lycopene.

Figure 8 indicates the relationship between concentrations of *cis*-lycopene and *all trans*-lycopene of *Lycopersicon esculentum* as follows:

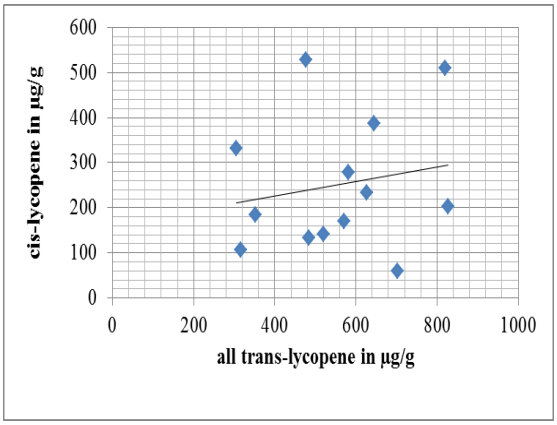

Figure 8: A graph of mass yield of *cis*-lycopene against mass yield of *all trans*-lycopene from *Lycopersicon esculentum.* Correlation = 0.182

From Figure 8, the levels of *all trans*-lycopene in *Lycopersicon esculentum* increased as the concentration of *cis*-lycopene increased. *All trans*-lycopene is anti-oxidant and precursor of vitamin A hence the increment trend of this carotenoid guarantees the nutrition benefit for intake of this fruit. Biological benefit of *cis*-lycopene in human body has not been investigated and well explained.

Figure 9 shows a graph of concentration of *cis*-lycopene against concentration of *all trans*-lycopene, both in µg/g, from *Lycopersicon esculentum.*

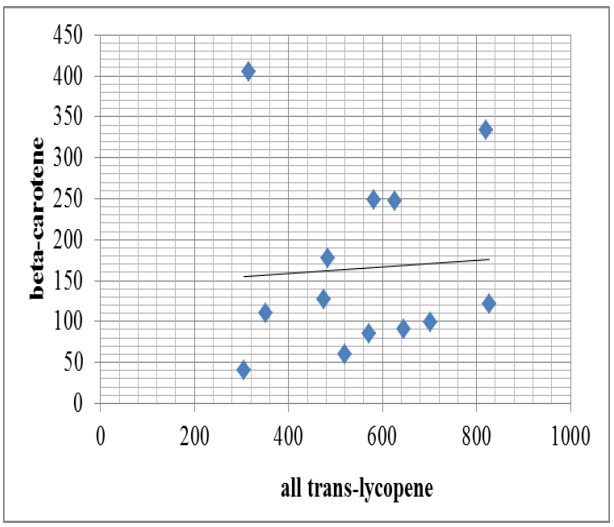

Figure 9: A graph of mass yield of β-carotene against mass yield of *all trans*-lycopene from *Lycopersicon esculentum*. Corelation = 0.0612

There was a slight increase of β-carotene with the increase of *all trans*-lycopene in *Lycopersicon esculentum*. Both β-carotene and *all trans*-lycopene are anti-oxidants and precursor of vitamin A hence the slight increment trend of these two carotenoid guarantee the nutrition benefit for intake of this fruit. Figure 10 shows the β-carotene and *cis*-lycopene correlation after extracted from tomato samples.

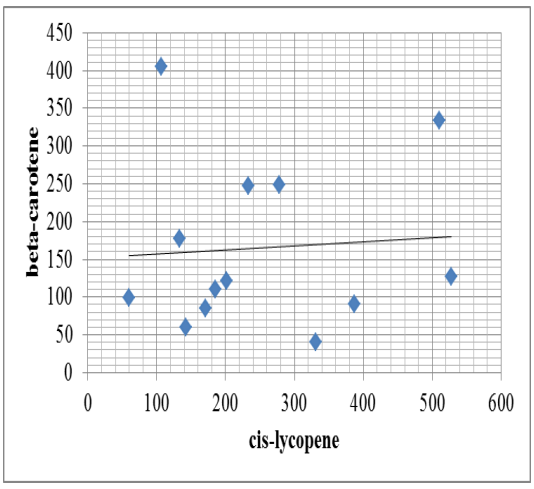

Figure 10: A graph of mass yield of β-carotene against mass yield of *cis*-lycopene from *Lycopersicon esculentum*. Correlation = 0.0709

From Figure 10, there was a slight increase of β-carotene with the increase of *cis*-lycopene in *Lycopersicon esculentum*. The increment trend of β-carotene, an ant-oxidant and precursor of vitamin A, guarantees the nutrition benefit for intake of this fruit.

4. Conclusion

The available tomatoes contain good amount of lycopene and β-carotene which are precursor of vitamin A, hence have high nutrients value. Amount of extracted lycopene and β-carotene from tomato varieties varies. The variation is caused by different conditions (14). Lycopene can undergo degradation when placed anywhere if not in conditions that stop degradation hence analysis of lycopene should be done immediately after extraction and separation. Individuals should be encouraged to use available fruits with high lycopene and β-carotene content such as tomatoes. The lycopene and β-carotene content should be considered in market value. Further studies should be done on investigation of *all trans*-lycopene, *cis*-lycopene and β-carotene in other fruits and vegetables including coloured sweet potatoes. It is far better and advisable to study and investigate the best conditions for growing tomatoes so as to contain the highest possible quantity of lycopene and β-carotene. Before extraction the tomato samples should be cooked in a little fat, such as olive oil, under high pressure and low temperature so as to break down the cell walls and make the fat-soluble lycopene more available.

4.1. Acknowledgement

I would like to thank Mr. Kassim and his father for their help and guide to get samples of tomatoes. I would also like to thank the State University of Zanzibar (SUZA) for its financial support of this study, SUZA Lab technicians for allowing me to use laboratory and to use some facilities, Zanzibar Chief Chemist for allowing using their balance for measuring the dried extracts of food nutrients.

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