Quantification of *All Trans*-Lycopene, *Cis*-Lycopene and Β-Carotene from Watermelons Found in Zanzibar

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Abstract

Red-fleshed watermelons (Citrullus lanatus) contain a high level of lycopene, and small amount of β-carotene which have been reported to have many important health benefits. However, very little is known on the quantity of all trans-lycopene, cis-lycopene and β-carotene found in watermelons grown in Zanzibar. The objective of this study was to quantify all trans-lycopene, cis-lycopene and β-carotene carried by watermelons available in Zanzibar Islands. Samples were collected from Mkokotoni area in Zanzibar, a representative of the Zanzibar Islands and extracted by using solvent system of hexane/acetone/ethyl acetate (4:2:1 v/v/v). The extracts were filtered and the lycopene layer separated from the filtrate, washed, dried by rotary evaporator and then dissolved in hexane. The concentrated hexane solution was then subjected to column chromatography on silica gel. The all trans-lycopene, cis-lycopene and β-carotene fractions were collected, dried by nitrogen gas and weighed. The dilute hexane solutions of all trans-lycopene, cis-lycopene and β-carotene were scanned using a UV-VIS spectrophotometer. The results obtained showed that mass (in μg/g) of all trans-lycopene varied from 93 to 1929, cis-lycopene varied from 67 to 827 and β-carotene varied from 25 to 881. The fruit contains considerable amounts/quantities of food nutrients beneficial to human health.

Key words: *All trans*-lycopene, *cis-lycopene*, β-carotene, quantification, determination, nutrient, watermelon

INTRODUCTION

Lycopene is carotenoid which gives the characteristic deep-red colour of ripe fruits and their products (Shi & Maguer, 2000). It occurs naturally in some red fruits and is a major component found in their serum (Kun *et al*., 2006). It also occurs naturally in vegetables, algae, fungi, watermelon, pink grapefruit, pink guava, papaya and apricots (Gerster, 1997). Lycopene is a C_{40}-carotenoid made up of eight isoprene units. The β-carotene, the yellow pigment of the carrot, is the isomer of lycopene. It is insoluble in water but soluble in benzene, chloroform, carbon disulphide, ether, petroleum ether and hexane. It is almost insoluble in methanol, cyclohexane and ethanol (Boileau *et al*., 2002). It is a very lipaphilic molecule, and therefore, it must be solubilised to be absorbed from food, first by emulsification in the gastra contents and then incorporation into mixed micelles in the duodenum as facilitated by bile acid, surfactants and lipases (During & Harrison, 2004).

Once ingested, lycopene must first be released from the food matrix before it is incorporated into mixed micelles. Micelles contain bile salts, cholesterol, and fatty acids from the meal and the amphilic nature of the micelles structure helps to keep the lipophilic nutrients soluble in the aqueous digesta (During & Harrison, 2004). The micelles approach the unstirred water layer of the apical side of the intestinal cells (enterocytes) and lycopene passively diffuses across the apical membrane (During & Harrison, 2004). Dietary lycopene contains *cis*- isomer and *all trans*- isomer (Boileau *et al*., 2002). Dietary *all trans*-lycopene has ability to reduce the risk of chronic diseases such as cancer and coronary heart disease (Kun *et al*., 2006).

In human health *all trans*-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 (Kun *et al*., 2006). Its presence in diet makes considerable interest as it exhibits a physical quenching rate constant with singlet oxygen, almost twice that of β-carotene (Shi & Maguer, 2000). 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 (Wer, 2001). Lycopene is a precursor of vitamin A **(**Atessahin *et al*., 2006) as well as β-carotene (Terlecki *et al*., 2014). Red-fleshed watermelon contains high quantity of lycopene, a red pigmented carotenoid with powerful antioxidant properties (Davis *et al*., 2007). The red watermelon is rich in lycopene and is a far better source of the carotenoid lycopene than tomatoes are (Raloff, 2002). Most people are not aware of the amount and importance of lycopene and β-carotene available in locally available fruits such as red-fleshed watermelon and hence do not value these fruits. The objective of this study was to separate and quantify *all trans*-lycopene, *cis*-lycopene and β-carotene from red fleshed watermelons available in Zanzibar Islands.

MATERIALS AND METHODS

Sample Collection

Samples of watermelons (*Citrullus lanatus*) were collected from Mkokotoni/Mtowapwani farm areas of Zanzibar north district.

Sample Preparation

Samples of red-fleshed watermelons were cut into smallest possible pieces and then ground into a paste using a mortar and pestle.

Sample Extraction

A sample of ground watermelon (100 g) was put in a beaker and extracted with 100 ml of ethyl acetate, acetone and hexane (1:2:4) solvent mixture. The extract was filtered and the filtrate 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 two filtrates were combined together. The lycopene layer was separated from filtrate by using a separating funnel and concentrated to lowest possible volume by evaporating the solvent under vacuum without heating (Tan, 2006). The lycopene-containing organic layer was separated from two layers of original extract by using a separating funnel, washed by using saturated sodium chloride solution, followed by 10% aqueous potassium carbonate and another portion of saturated sodium chloride solution. This was followed by drying with anhydrous magnesium sulphate (Tyman, 1997).

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

A piece of cotton wool was placed at the bottom of a glass column followed by hexane (5ml) and clean sand (1cm). Activated silica (13 g – 14 g) was then mixed with hexane to make thick but pourable slurry and then poured carefully into the column. After settling, slurry of sand and hexane was added at the top of the column to a length of about 0.5 cm (Tan, 2006).

The concentrated extracts were introduced into the packed column and eluted with hexane. Total volume of hexane used in elution was not recorded. β-carotene was collected in a small beaker. Elution solvent of 15% – 20% (v/v) acetone in hexane was then added to the top of the column so as to accelerate the movement of orange-red (*all trans*-lycopene) band. This was collected into another container. The slow moving yellow-orange (*cis*-lycopene) band was also collected. The fractions were dried by using nitrogen gas and then weighed.

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). The cuvette was then emptied and filled three-quarter way with 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 (Chemat-Djenni *et al*., 2013 & Takehara *et al*., 2013).

RESULTS AND DISCUSSION

Mass of *All Trans*-Lycopene, *Cis*-Lycopene and Β-Carotene
The concentration of *all trans*-lycopene in watermelon (*Citrullus lanatus*) varied from 93 μg/g to 1929 μg/g of sample with average concentration of 521.3 μg/g and standard deviation of 525. Mass of *cis*-lycopene varied from 67 μg/g to 827 μg/g with average concentration of 214.4 μg/g and standard deviation of 68.4. Mass of β-carotene varied from 25 μg/g to 881 μg/g with average concentration of 231 μg/g and standard deviation of 232 (Table 1).

Comparison of the determined *all trans*-lycopene, *cis*-lycopene and β-carotene in this study with those reported in the literature show that other studies have reported watermelon juice as containing 20000 μg/g lycopene, the juice contains 18800 μg/g (94%) *all trans*-lycopene and 1200 μg/g (6%) *cis*-lycopene (Collins *et al.*, 2004). Another study reported that lycopene extracted from *Citrullus lanatus* contains 88% *all trans*-lycopene and 12% *cis*-lycopene (Katherine, 2008). In another study the mass of lycopene and β-carotene in watermelon (seedless) was found to be 6.5 ± 0.1 μg/g and 0.1 ± 0.06 μg/g respectively (Barba *et al.*, 2005). Variety and growing environment are among the causes of the differences (Mayeaux, 2006). Other studies have reported higher lycopene content in watermelons than in this study.

Concentration of *all trans*-lycopene of 521 μg/g of sample of watermelon sampled from Mkokotoni, Zanzibar < concentration of *all trans*-lycopene of 18,800 μg/g of sample of watermelon from Spain (Barba *et al.*, 2006). Concentration of *cis*-lycopene of 214 μg/g of sample of watermelon sample from Mkokotoni, Zanzibar < concentration of *cis*-lycopene of 1,200 μg/g of sample of watermelon sampled from Spain (Barba *et al.*, 2006). Concentration of β-carotene of 231 μg/g of sample of watermelon from Mkokotoni, Zanzibar > concentration of β-carotene of 0.1 μg/g of sample of watermelon (seedless) sampled from Spain (Barba *et al.*, 2006).

Table 1: Mass of *all trans*-lycopene, *cis*-lycopene and ß-carotene extracted from watermelons (*Citrullus lanatus)*

Sample used		Mass of sample (g)	Mass (g)			Concentration(µg/g of sample)		
			All trans lycopene	*Cis* lycopene	β-carotene	*All trans* lycopene	*Cis* lycopene	β-carotene
Citrullus	1	100	0.0447	0.0067	0.0304	447	67	304
lanatus	2	100	0.0580	0.0089	0.0025	580	89	25
	3	100	0.1929	0.0174	0.0881	1929	174	881
	4	100	0.0886	0.0146	0.0175	886	146	175
	5	100	0.0200	0.0827	0.0101	200	827	101
	6	100	0.0164	0.0106	0.0176	164	106	176
	7	100	0.0135	0.0261	0.0127	135	261	127
	8	100	0.0514	0.0104	0.0139	514	104	139
	9	100	0.0265	0.0386	0.0082	265	286	82
	10	100	0.0093	0.0084	0.0304	93	84	304
Average	1	100				521.3 (53.9%)	214.4 (22.2%)	231.4 (23.9%)
Variance						275288	4673	54013
Std deviation						525	68.4	232

***All trans*-lycopene, *cis*-lycopene and β-carotene Content in Watermelons**

The study has shown that the quantity of *all trans*-lycopene is higher compared to that of *cis-l*ycopene. The quantity of β-carotene obtained is higher compared to that of *cis*-lycopene. Both lycopne (especially *all trans*-lycopene) and β-carotene are antioxidants and are precursors of vitamin A.

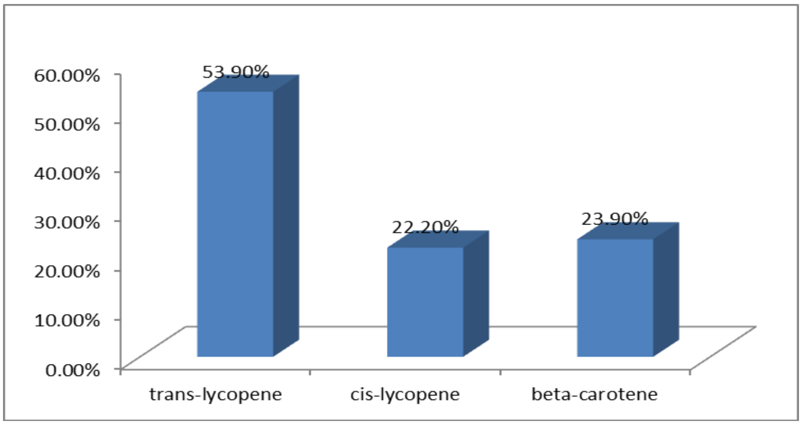
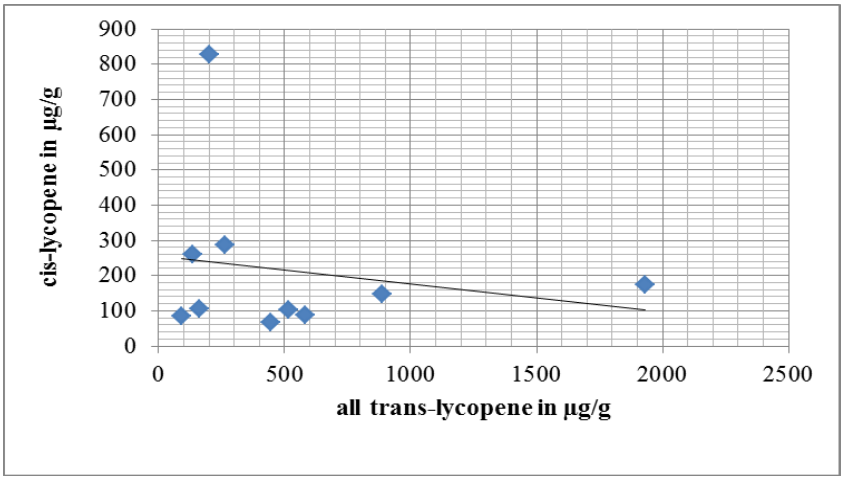


Figure 1: Percentage concentration of *all trans*-lycopene, *cis*-lycopene and β-carotene from watermelon

Correlations of *all trans*-lycopene, *cis*-lycopene and β-carotene

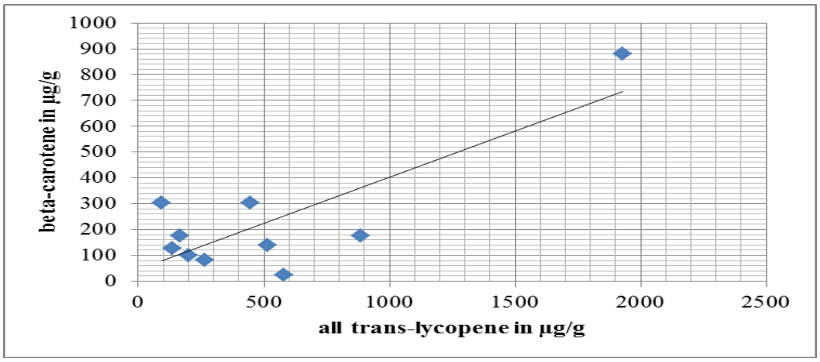
The concentration of *cis*-lycopene in watermelon decreased with the increase in concentration of *all trans*-lycopene (Figure 2).The graph shows that the concentration of *cis*-lycopene in watermelon somehow decreased with the increase of *all trans*-lycopene. The trend has beneficial importance of intake of fruits since *all trans*-lycopene is attributed to anti-oxidant activity and other lycopene benefits rather than *cis*-lycopene. The trend also shows that when the level *all trans*-lycopene is high the level of *cis*-lycopene is low simultaneously and vice-versa. The importance of this figure is to show the composition of these two carotenoids in biometabolic product.



Correlation (R) = - 0.19046

Figure 2: A graph of concentration of *cis*-lycopene against concentration of *all trans*-lycopene of watermelon

Correlation of β-carotene and *all trans*-lycopene extracted from watermelon is shown in figure 3 below.

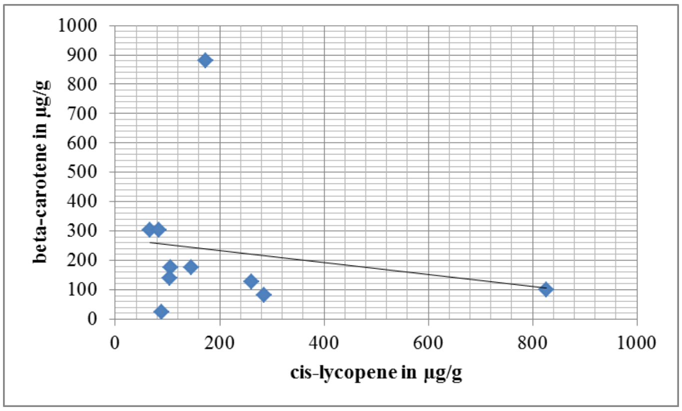


Correlation = 0.809

Figure 3: A graph of concentration of β-carotene against concentration of *all trans*-lycopene of watermelon

The concentration of β-carotene increased with the increase of concentration of *all trans*-lycopene in watermelon samples (Figure 3). Both β-carotene and *all trans*-lycopene are anti-oxidants and precursor of vitamin A hence the increasing trend of these two carotenoids guarantee the nutrition benefit for intake of these fruits (watermelons). Beta-carotene is an isomer of lycopene which is produced naturally as *all trans*-lycopene. The increase of concentration of *all trans*-lycopene will cause also the increase of concentration of beta-carotene.

There was a slight decrease of β-carotene in watermelon samples with the increase of *cis*-lycopene (Figure 4).



Corelation = - 0.191

Figure 4: A graph of concentration of β-carotene against concentration of cis-lycopene of watermelon

On the other hand the trend also shows the increase of β-carotene with decrease of *cis*-lycopene and hence the increment trend of β-carotene, an anti-oxidant and precursor of vitamin A, guarantee the nutrition benefit for intake of this fruit. The biosynthesis of lycopene in eukaryotic plants and in prokaryotic cyanobacteria begins with mevalonic acid, which is converted into dimethylallyl pyrophosphate**.** This is then condensed with three molecules of isopentenyl pyrophosphate (an isomer of dimethylallyl pyrophosphate), to give the twenty carbon geranyl geranyl pyrophosphate. Two molecules of this product are then condensed in a tail-to-tail configuration to give the forty carbon phytoene, the first committed step in carotenoid biosynthesis. Through several desaturation steps, phytoene is converted into lycopene. The two terminal isoprene groups of lycopene can be cyclized to produce β-carotene (Gerster, 1997). *Cis*-lycopene is produced from degradation of *all trans*-lycopene when exposed to light after being produced naturally (Cheynier, 2005).

Figure 5 below shows the characteristics of *all-trans* lycopene and *cis*-lycopene when scanned in UV-VIS. It shows the finger print characteristics of *all trans*-lycopene and *cis*-lycopene with three peaks of maximum absorption spectrum for each:

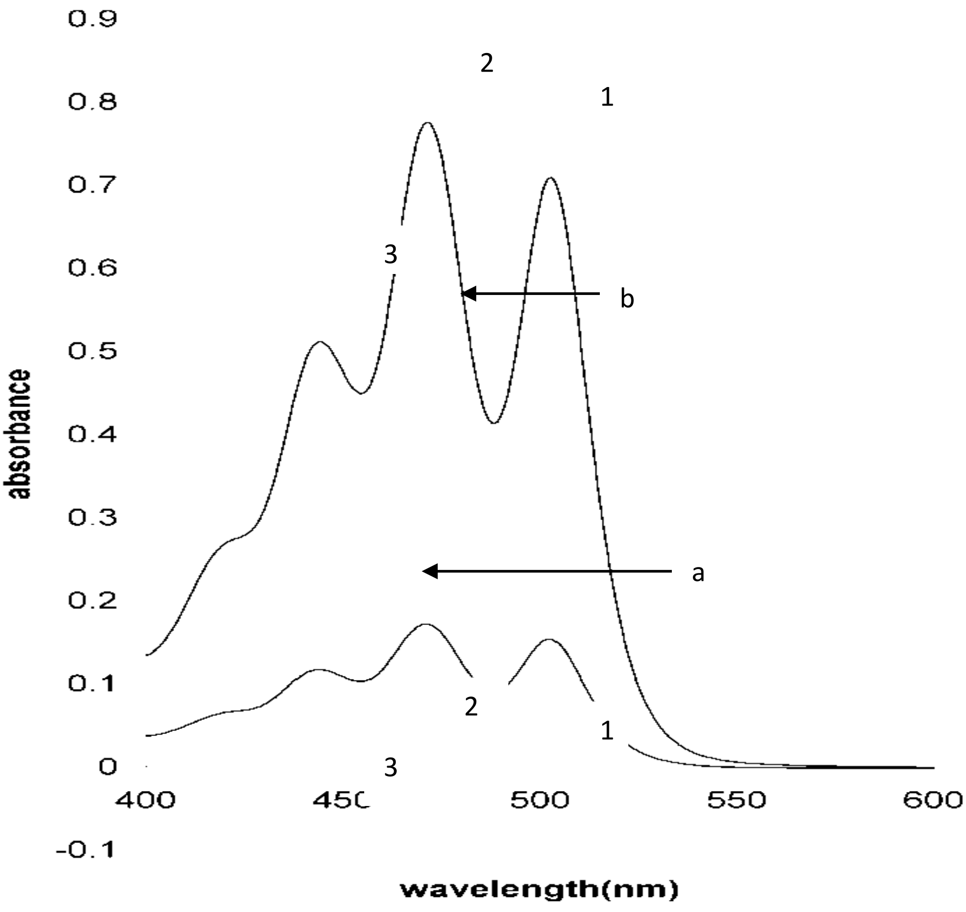


Figure 5: Normal derivative of absorption spectra characteristics of *all trans*-lycopene (b) and *cis*-lycopene (a) extracted from watermelon. Concentration of lycopene scanned in UV-VIS spectrophotometer was 0.015g/litre in hexane solution.

In b $\lambda_{1\ max}$ = 503nm, $\lambda_{2\ max}$ = 473nm and $\lambda_{3\ max}$ = 444nm.
In a $\lambda_{1\ max}$ = 502nm, $\lambda_{2\ max}$ = 471nm and $\lambda_{3\ max}$ = 444nm

CONCLUSION AND RECOMMENDATIONS

The locally available fruits contain good amounts of lycopene and β-carotene which are precursors of vitamin A, therefore have high nutrients value. Quantity of extracted lycopene and β-carotene from watermelons varies. The variation is caused by different conditions (Caris-Veyrat & Genard, 2008). Lycopene can undergo degradation when placed anywhere if not in conditions that stop degradation (Gerster, 1997). Analysis of lycopene should be done immediately after extraction and separation. People should be encouraged to use available fruits in general and watermelons specifically with high lycopene and β-carotene content. The lycopene and β-carotene content should be considered in market value. Other fruits and crops should be investigated to determine their lycopene and β-carotene content. Studies should also be carried out to determine the best conditions for growing watermelons with the highest possible content of lycopene and β-carotene.

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