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RESEARCH ARTICLE

QUANTIFICATION OF LYCOPENE EXTRACTED FROM WATERMELON AND TOMATO VARIETIES FROM NAKULABYE MARKET IN KAMPALA, UGANDA

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ABSTRACT

Tomatoes *(Solanum lycopersicum)* and red-fleshed watermelons *(Citrullus lanatus)* contain a high level of lycopene, which has been reported to have many important health benefits. Watermelon tomato varieties are available plentifully in Uganda, however, little information existing in Uganda concerning the quantity of lycopene in watermelon and tomato varieties. The objective of this study was to separate and quantify lycopene from selected watermelon and tomato varieties from Uganda. The varieties were bought from Nakulabye market in Kampala**,** 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 fractionated by using an alumina column chromatography. The lycopene fractions were collected, dried by using nitrogen gas and then weighed by chemical balance**.** The solutions of lycopene in hexane were scanned by using UV-VIS spectrophotometer. The results obtained showed that the quantity of lycopene varies from140 µg/g to 3400 µg/g wet weight in tomato and from 326 µg/g to 1670 µg/g in watermelon**.** The variation is caused by different conditions. Both tomato varieties and watermelons contain appreciable quantity of lycopene necessary for daily food intake. It is advisable to investigate the best conditions for growing tomatoes and watermelons so as to contain the highest possible quantity of lycopene.

INTRODUCTION

Lycopene is a pigment which is principally responsible for the characteristic deep-red colour of ripe tomato fruits and tomato products (Shi & Maguer, 2000). It occurs naturally in tomato as a carotenoid and is a major component found in serum (Kun *et al*., 2006). It also occurs naturally in certain fruits, vegetables, algae and fungi. Other significant sources are watermelon, pink grapefruit, pink guava, papaya and apricots (Gerster, 1997). 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. Lycopene is an unsaturated hydrocarbon with chemical name of 2,6,10,14,19,23,27,31-octamethyl-2, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 30-dotriacontriacontatridecaene. Its molecular weight is 536.9 (Gerster, 1997). Dietary lycopene has ability or potential to reduce the risk of chronic diseases such as cancer and coronary heart disease (Kun *et al*., 2006). 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 (Kun *et al*., 2006). 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 (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). 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. Red-fleshed watermelon (such as *Citrullus lanatus),* 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). Cooking the tomato in a little fat, such as olive oil, breaks down the cell walls and makes the fat-

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soluble lycopene more available (Everson *et al,* 2004). Most of people are not aware of the amount and importance of lycopene available in local available fruits such as tomatoes and red-fleshed watermelon and hence do not give full respect of these fruits. Because of lack of awareness food staff in Uganda are fortified with vitamin A (Kawuma, 2002). The objective of this study was to separate and quantify *lycopene* from selected *watermelon* and *tomato* varieties from Uganda.

MATERIALS AND METHODS

Apparatus

Beakers, conical flasks, measuring cylinders, dropper, rotary evaporator and glass column separation for chromatography, analytical balance, UV-1700 CE Spectrophotometer (Shimodzu, Kyoto, Japan), for analysing lycopene samples.

Chemicals

Alumina (chromatography grade), saturated aqueous solution of sodium chloride, 10% potassium carbonate aqueous solution, anhydrous magnesium sulphate, Acetone, hexane, ethanol, ethyl acetate, pet-ether, magnesium sulphate, sodium chloride and potassium carbonate. Nitrogen gas was used for drying lycopene extracts.

Sample Collection

Samples of tomato and watermelon fruits were bought from Nakulabye market in Kampala. The samples were usually processed immediately because of lack of sufficient storage facilities.

Tomato fruit varieties

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

Watermelon (Citrullus lanatus)

Red fleshed watermelons with green coat.

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. A mass of 30 g, 50 g and 215 g of each variety were measured and extracted. The reason of changing mass of sample was to try to extract more lycopene for analytical purposes.

Sample Extractions

A sub sample of 30 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 16 ml:32 ml: 64 ml. 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. The filtrate was concentrated to lowest possible volume by evaporating the solvent under vacuum (Tan, 2006). A sample of 30 g of flesh of watermelon was also grounded, extracted using the same solvent mixture and then concentrated (Collins *et al,* 2004). The same procedure was repeated for 50 g mass of sample. For 215 g mass of sample the volume used was 32 ml: 16 ml: 8 ml. Before a chromatographic separation, the lycopene-containing organic layer was separated from two layers of original extract 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 then dried with anhydrous magnesium sulphate (Morris *et al*, 1994).

Preparation of a Column

Concentrated crude extracts were subjected to column chromatography. The column chromatography packed with alumina was used. A chromatography column was mounted at a retort stand vertically. A small amount of cotton was placed at the bottom of column. The cotton was fixed down by using long glass rod and the stop cork was closed. Hexane (5 ml) was added and air bubbles were taped out. Clean sand (1 cm) was added to the column. The solvent column level was above the sand level. Any trapped air was removed once again by tapping on the column. Then, activated alumina (13 g – 14 g) was mixed with hexane to make thick but pourable slurry. The slurry was mixed well to suspend the alumina, and then poured carefully into the column. The length of Alumina column was about 15 cm - 17 cm. The trapped air bubbles at this point were removed, as air bubbles retained in the column would reduce separation efficiency. Alumina was allowed to settle, and then the solvent was allowed to run at a slow rate from the bottom of column until the solvent level was 2 cm – 3 cm above the top of alumina bed. Slurry of sand and hexane was prepared and added at the top of the column with length of about 0.5 cm. The solvent was slowly allowed to drip from the bottom of the column until its level was just above the top of the sand (Tan, 2006).

Separation

Column chromatographic separation technique was used to separate lycopene from other carotenoids. The concentrated extracts were put to the top of the alumina column and eluted with hexane. The solvent was allowed to run from the bottom of alumina column to the beaker until its level was just above the alumina bed. Then carefully some additional clean hexane solvent was introduced to produce ~ 10 cm of height above the alumina bed. Hexane solvent was then allowed to run slowly through the alumina column with constant monitoring of the solvent height and elute was collected in the beaker. An orange band started migrating down the column but stopped at approximately 1cm from the top. The yellow (β-carotene) pigment continued moving down the column. When the cotton turned yellow, the portion containing β-carotene was collected in a small beaker. When the entire yellow band had left the column, the elution solvent of 15% – 20% (v/v) acetone in hexane was added to the top of the column. This solvent accelerated the motion of orange-red (lycopene) band and hence elute was collected into another container from the bottom of the column. The slow moving yellow-orange band was also collected. The solvents of all collected extracts (fractions) were concentrated on a rotary evaporator. Stream of nitrogen gas was used for drying the extracts collected and the

weight of the dry lycopene was measured. The extracts were taken for spectral analysis as soon as possible. The melting point of lycopene was measured by using the capillary tube attached to a thermometer and then immersed in paraffin oil. The oil was heated on a bunsen burner till the lycopene crystal in tube started to melt and hence the temperature recorded.

Analysis of Lycopene

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 (Wrolstad, 2005).

Quantitative analysis

Concentrations (mass) of lycopene extracted were measured by using analytical balance. The quantities obtained were recorded and converted into microgram per gram of sample (µg/g).

RESULTS AND DISCUSSION

Level of lycopene extracted: Concentrations of lycopene (µg/g wet weight) extracted in different samples were presented in Table 1. In watermelon (*Citrullus lanatus*) concentration of lycopene varied from 326 µg/g to 1670 µg/g of sample with average concentration of 998 µg/g and standard deviation of 475. 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 279 µg/g to 3330 µg/g of sample and its average concentration was 1482 µg/g. Number of µg of lycopene per gram of fresh sample in *Solanum lycopersicum cerasiforme* ranged from 140 µg/g to 3400 µg/g while that of *Solanum quitoense* was between 333 µg/g and 2670 µg/g of sample. Their average concentrations were 1470 µg/g and 1281 µg/g for *Solanum lycopersicum cerasiforme* and *Solanum quitoense*, respectively. Their standard deviations were 1038, 1242 and 876 respectively. Comparison of the determined lycopene in this study with those reported in the literature show that other studies are reporting much higher concentration in tomato (8800 µg/g – 42000 µg/g)] and watermelon [(23000 µg/g – 72000 µg/g)] wet weight (Gerster, 1997) than those determined in tomato (140 µg/g – 3400 µg/g) wet weight and watermelon (326 µg/g – 1670 µg/g) wet weight in this study.

Table 1. Quantities of lycopene extracted from watermelon and tomato samples

Sample used	S/N	Mass of sample in g	Mass of lycopene in g	Mass of lycopene in µg/g of sample	Mean (average)	Variance	Std deviation
Citrullus lanatus	1	30	0.03	1000	998	225794	475
	2	30	0.05	1670			
	3	50	0.05	1000			
	4	215	0.07	326			
Lycopersicon esculentum	1	30	0.04	1330	1482	1077177	1038
	2	30	0.05	1670			
	3	30	0.10	3330			
	4	50	0.04	800			
	5	215	0.06	279			
Solanum lycopersicum cerasiforme	1	30	0.02	667	1470	1544652	1242
	2	30	0.05	1670			
	3	50	0.17	3400			
	4	215	0.03	140			
Solanum quitoense	1	30	0.04	1330	1281	767632	876
	2	30	0.08	2670			
	3	215	0.17	791			
	4	30	0.01	333			

Comparison of lycopene content in watermelon and tomato samples is shown in the following Figure 1

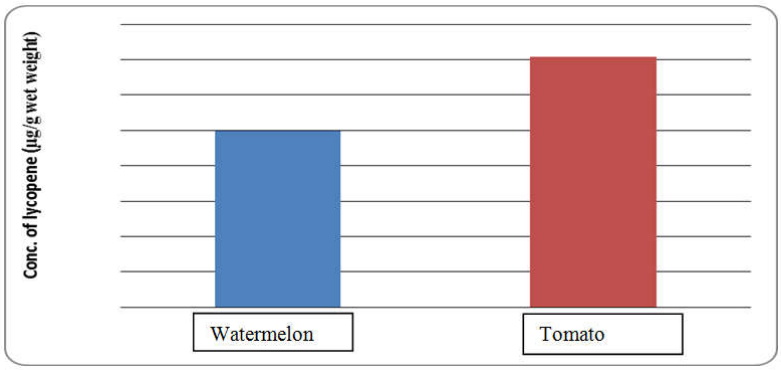


Figure 1. Lycopene content in watermelon and tomato samples

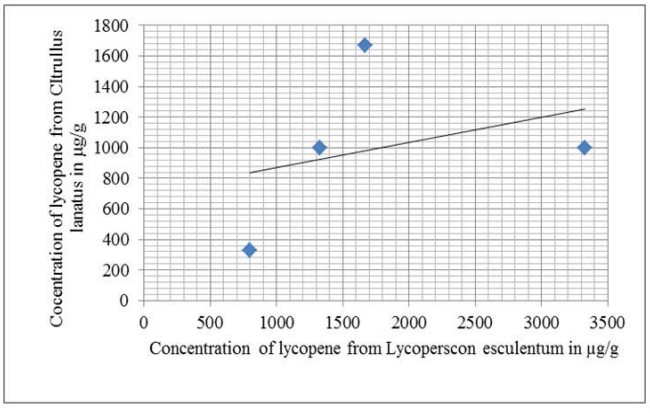
Also, lower concentrations of lycopene were reported from other studies. One study reported that level of lycopene in mini watermelons were ranging from 67 µg/g to 96 µg/g fruit weight (Collins *et al,* 2004). Another research done in University of Madrid (Spain) reported mass of lycopene in watermelon (seedless) was 6.5 ± 0.1 µg/g (Barba *et al.*, 2006). Variety and growing environment are among the causes of the difference (Mayeaux, 2006). Contrary to this study, literature reported higher lycopene content in watermelon than in tomatoes, regardless of higher water content in watermelon compared to tomato.

Lycopene content in fruits: In general the study has shown that the lycopene content (µg/g wet weight) in tomato is relatively higher compared to watermelon (Figure 1). This was probably attributed by higher water content in watermelon compared to tomato. In this regard the lycopene content on the basis of dry mass samples was likely to be higher in watermelon than tomato. The observed variations were attributed to the quality of the fruits (May –July) were of higher quality than those from (September – November). The time taken for sample from sampling to analysis was longer for those from May –July (more than 40 days) than those from September – November (less than 40 days). Though the extracted lycopene was kept in hexane solution it seemed that the degradation of lycopene had occurred. This could be due to the fact that the stored temperature did not reach -20°C and it was not in a vacuum space, good conditions for controlling degradation of lycopene **(**Gerster, 1997).

The melting point of lycopene: The measured melting point of lycopene obtained from this research was 170°C - 172°C. From literature the melting point of lycopene is 172°C –175°C **(**Gerster, 1997). The result is quite close to that in literature and the short deviation might be caused by the error of measurement due to the differences in apparatus used and the sensitivity of thermometer and graduated scale. The deviation might also be caused by the different in environment, the external atmospheric pressure and temperature, and degree of purity of lycopene.

Correlations of lycopene from watermelon and tomato varieties

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

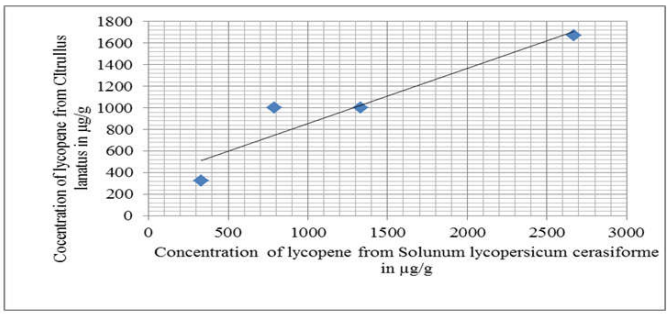


Correlation = 0.326

Figure 2. Correlation of lycopene concentration from *Citrullus lanatus* and *Lycopersicon esculentum*

The graph shows that the concentration of lycopene extracted from watermelon (*Citrullus lanatus*) increased with the increase concentration of *Lycopersicon esculentum*. Since the variation is positively increased it seemed to what extent living bodies can benefit positively to these fruits.

From Citrullus lanatus and Solanum lycopersicum cerasiforme: The correlation of level of lycopene extracted from *Citrullus lanatus* and *Solanum lycopersicum cerasiforme* is well shown in following Figure 3:



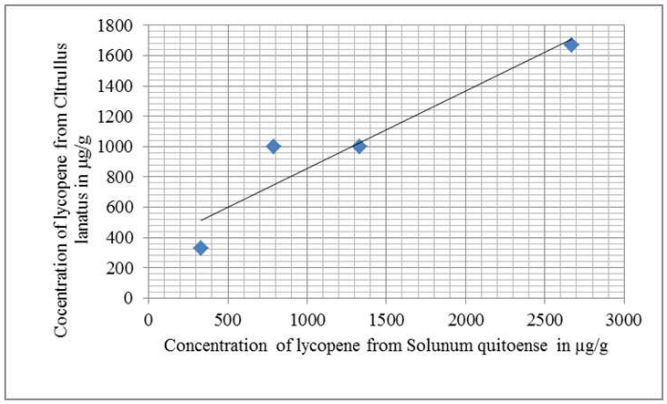
Correlation = 0.436

Figure 3. Correlation of lycopene concentration from *Citrullus lanatus* and *Solanum lycopersicum cerasiforme*

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 nutrient (lycopene) contents.

From *Citrullus lanatus* and *Solanum quitoense*

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



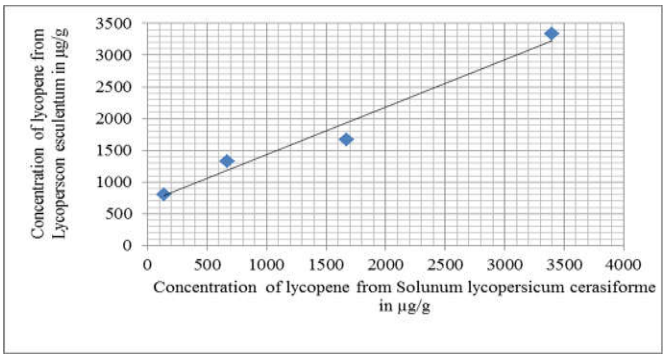
Correlation = 0.943

Figure 4. Correlation of lycopene concentration from *Citrullus lanatus* and *Solanum quitoense*

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 nutrient (lycopene) content.

Correlations of lycopene concentration within tomato varieties

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 5.



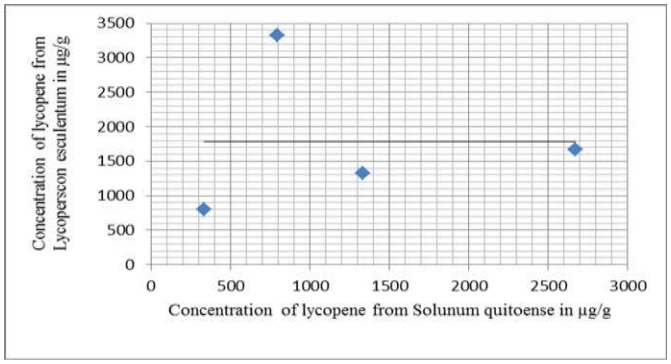
Correlation = 0.986

Figure 5. Correlation of lycopene concentration from *Lycoperscon esculentum* and *Solanum lycopersicum cerasiforme*

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 to nutrient (lycopene) contents. It also seems to what extent the varieties of tomato are nearly in similarity.

Between Lycoperscon esculentum and Solanum quitoense

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

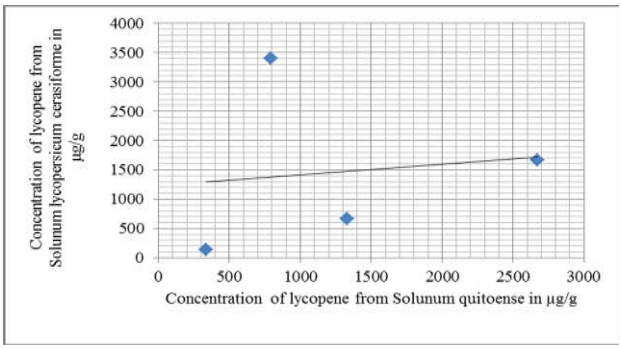


Correlation = -0.0016

Figure 6. Correlation of lycopene concentration from *Lycoperscon esculentum* and *Solanum quitoense*

The above diagram shows that correlation between two parameters is nearly nil.

Between Solanum lycopersicum cerasiforme* and *Solanum quitoense: The following Figure 7 shows relationship between levels of lycopene extracted from *Solanum lycopersicum cerasiforme* and *Solanum quitoense* as shown below:



Correlation = 0.127

Figure 7. Correlation of lycopene concentration from *Solanum lycopersicum cerasiforme* and *Solanum quitoense*

The correlation in the above graph shows that the concentration of lycopene extracted from *Solanum lycopersicum cerasiforme* increased slightly 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 nutrient (lycopene) contents.

Conclusion

The local available fruits contain good amount of lycopene and therefore have high nutrients value. Quantity of extracted lycopene from both tomatoes and watermelons varied due to different conditions. Lycopene can undergo degradation when placed anywhere if not in conditions that stop degradation as explained in literature. Analysis of lycopene should be done immediately after extraction and separation.

Recommendations

Society should be encouraged to use available fruits with high lycopene content. The lycopene content should be considered in market value. Individuals should encourage using and drink tomato and watermelon juice since the process take place in normal temperature with least degradation compared with other processes. Further studies should be done on investigation of lycopene in other fruits and crops. It is far better and advisable to study and investigate the best conditions for growing tomatoes and watermelons so as to contain the highest possible quantity of lycopene.

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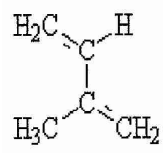
Appendix

Chemical structure of isoprene unit



Isoprene unit

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

Characteristics of lycopene

When solution of lycopene was run in UV/VIS Spectrophotometer it gave characteristics with three maximum peaks of absorption spectrum as shown below for both watermelon and tomato extracts

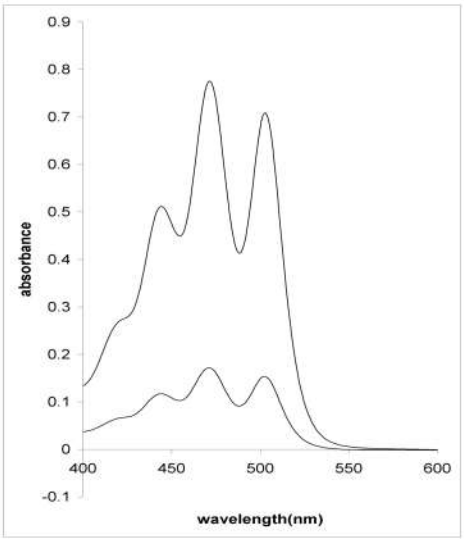


Figure 8. Normal derivative of absorption spectra characteristics of lycopene from red-orange band (b) and lycopene from yellow-orange band (a) extracted from watermelon. Concentration of lycopene scanned in UV-VIS spectrophotometer was 0.015g/litre in hexane solution

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

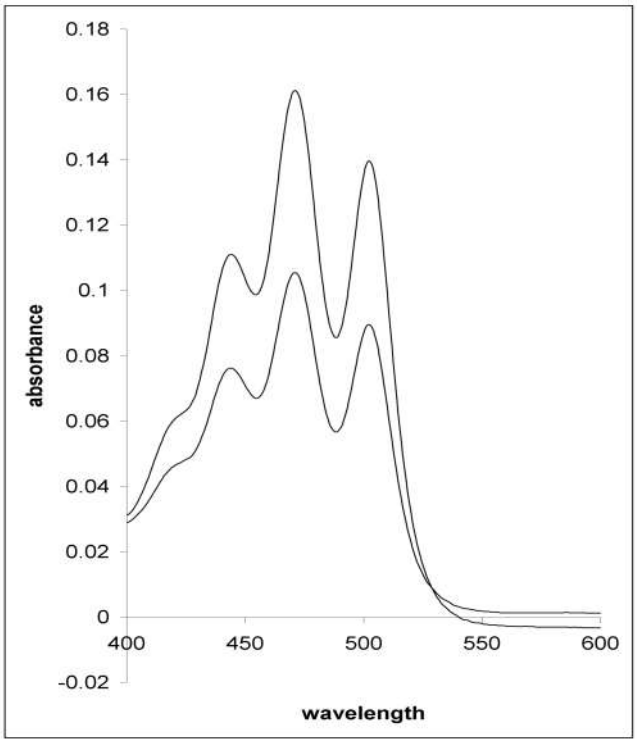


Figure 9. Normal derivative of absorption spectra characteristics of lycopene from red-orange band (b) and lycopene from yellow-orange band (a) extracted from tomato. Concentration of lycopene scanned in UV-VIS spectrophotometer was 0.01g/litre in hexane solution for each of the two

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

λ_{max} = wavelength of maximum peak of absorption spectrum.

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