ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 9, Issue, 11, pp.60322-60328, November, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

DEGRADATION OF LYCOPENE, FROM *ALL TRANS*-LYCOPENE TO *CIS*-LYCOPEN, BY HEATING

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ARTICLE INFO

Article History:
Received 16th August, 2017
Received in revised form
21st September, 2017
Accepted 10th October, 2017
Published online 30th November, 2017

Key words:
Citrullus lanatus,
Tomato,
All trans-lycopene,
Cis-lycopene,
Wavelength,
Peak,
Spectra.

ABSTRACT

Red tomatoes and red-fleshed watermelons contain a high level of lycopene with two isomers, *cis*- and *all trans*- isomers. *All trans*-lycopene is more valuable nutrient than *cis*-lycopene. A high content of *cis*-lycopene lowers the dietary value of fruit or the fruit - products. Individuals eat food staff with lycopene contents without awareness whether lycopene has been degraded or not. However, there is no researched information existing so far in Uganda to establish the dietary value of lycopene before and after heating. The objective of this study was to isolate *all trans*-lycopene and *cis*-lycopene and then to measure their maximum peaks of characteristic before and after degradation by heat from selected tomato and watermelon varieties from Uganda. Hexane solutions of *cis*-lycopene and *all trans*-lycopene were scanned by using UV - VIS spectroscopy. The results obtained showed that the UV - VIS absorption spectra of *all trans*-lycopene and cis-lycopene extracted from *Citrullus lanatus* has three peaks with maximum wavelengths of (503, 473 and 444 nm) and (502, 471 and 444 nm) respectively. When heated at 74^0C for 2 hours their maximum wavelengths were (501, 469 and 443 nm) for all trans-lycopene and (500, 469 and 444 nm) for *cis*-lycopene. When heated again at 74^0C for 2 hours their maximum wavelengths were (502, 470 and 444 nm) for both *all trans*-lycopene and *cis*-lycopene and hence lycopene has been completely degraded by heating. The absorption spectra characteristics of *all trans*-lycopene and *cis*-lycopene from tomato had three peaks with maximum wavelengths of (502, 472 and 444 nm) and (502, 471 and 444 nm) respectively. When heated at 74^0C for 2 hours their maximum wavelengths were (500, 469 and 443 nm) for both *all trans*-lycopene and *cis*-lycopene and hence lycopene has been completely degraded by heating. The significance of this result is that *all trans*-lycopene from watermelon takes longer time to be totally converted to *cis*-lycopene by heating rather than *all trans*-lycopene from tomato and hence is more stable to undergo degradation and losing its quality by heat. This gives more a preference of watermelon for dietary purpose to tomato.

Citation: Haji Khamis, Kituyi Lusweti, Haji Mwevura, Steven Nyanzi and Kiremire, 2017. "Degradation of lycopene, from *all trans*-lycopene to *CIS*-lycopen, by heating", *International Journal of Current Research*, 9, (11), 60322-60328.

INTRODUCTION

Lycopene is a pigment which is principally responsible for the characteristic deep-red colour of ripe tomato fruits and tomato products **(**Gerster, 1997). It occurs naturally in certain fruits such as tomato and watermelon as a carotenoid (Kun*et al.,* 2006). Dietary lycopene has two isomers, *cis*- and all *trans*-isomers but *all trans*-lycopene is more valuable nutrient than *cis*–lycopene. Dietary lycopene has ability to reduce the risk of chronic diseases such as cancer and coronary heart disease (Kun*et al.,* 2006). It plays the role of an antioxidant and has beneficial properties to other mechanisms such as intercellular gap junction communication (Kun *et al.*, 2006). Dietary lycopene or other phytochemicals consumed as oil based tomato products confer cardiovascular benefits (Sesso *et al.*, 2003). Undesirable degradation of lycopene not only affects the sensory quality of the final products, but also the health benefit of tomato-based foods for the human body (Shi and Maguer, 2000). Isomerization and oxidation are the main causes of degradation during processing. Isomerization converts *all trans*-isomers to *cis*-isomers. *Cis*- isomers increase with temperature and time taken for processing (Shi and Maguer, 2000). Increasing the temperature from 21 C to 26^0C reduces total carotene content without affecting lycopene content of fruit. Further increase in temperature from 27^0C to 32 C reduces ascorbate, lycopene and its precursor's contents (Caris-Veyrat and Genard, 2008). Bioavailability (absorption) of lycopene of *cis*-isomers in food is higher than that of *all trans*-isomers. Local consumers eat food containing lycopene without knowing the quality and grade of lycopene they intake from feeding fruits and vegetables. Probably, most lycopene

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present in food staff become degraded due to high temperature used during cooking. Development of lycopene-rich food, as well as food-grade and pharmaceutical-grade lycopene is needed by consumers from the food industry of high quality lycopene products that meets food safety regulations (Shi and Maguer, 2000). Literature does not explain which fruit has a better quality of lycopene which do not degraded easily during heating. The objective of this study was to isolate and examine degradation of lycopene caused by heating, from selected tomato and watermelon varieties from Uganda.

MATERIALS AND METHODS

Sampling

Watermelon and tomato fruits were bought from Nakulabye market in Kampala, Uganda and processed as quickly as possible.

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. 500 g mass of each variety were used in extraction of lycopene.

Sample extractions

500 g of grounded tomato and watermelon was each put in a separate beaker and extracted with a mixture of ethyl acetate, acetone and hexane (1:2:4). The extract was filtered and the filtrate was placed into a second beaker. The filtrate was concentrated to lowest possible volume by evaporating the solvent under vacuum or stream of nitrogen without heating (Tan, 2006). Before a chromatographic separation, the lycopene-containing organic layer was separated from two layers of original extract by using separating funnel, washed by using saturated sodium chloride solution, followed by 10% aqueous potassium carbonate and another portion of saturated sodium chloride solution and then dried with anhydrous magnesium sulphate (Morris *et al.,* 1994).

Preparation of a 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 alumina (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 (Tan, 2006).

Separation of All trans-lycopene and *cis*-lycopene

The concentrated extracts were put to the top of the alumina column and eluted with hexane. β-carotene 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 (lycopene) band and collected into another container. The slow moving yellow-orange band was also collected. Yellow-orange band is cis-lycopene and orange-red band is all trans-lycopene (Basu and Lmrhan, 2007). The extracts were dried by using a stream of nitrogen gas and then weighed. 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 till the lycopene crystal in tube started to melt and hence the temperature recorded.

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). Both *all trans*-lycopene and *cis*-lycopene extracted from watermelon and tomato were scanned in UV-VIS spectrophotometer. Their wavelengths of maxim peaks from each characteristics were read by using Microsoft Excel and recorded.

Quantitative measurement

Mass of both all trans-lycopene and cis-lycopene were measured by using chemical balance and recorded. Their quantities of matter were then converted into micro gram per gram of sample.

Heating hexane solution

The hexane solutions of all trans-lycopene , cis-lycopene from watermelon in test tube separately, and the hexane solution of all trans-lycopene and cis-lycopene from tomato in test tube also separately were put in beaker with water and heated by boiling water in water bath for two hours. The solutions were left to cool and then each scanned in UV-VIS spectrometer. The wavelengths of three maximum peaks from each characteristics were read by using Microsoft Excel and recorded. Heating were repeated again for two hours and the hexane solutions were scanned in UV-VIS after cooling. The wavelengths of three maximum peaks from each characteristics were also read by using Microsoft Excel and recorded.

RESULTS AND DISCUSSION

Mass of all trans-lycopene and cis-lycopene extracted

Mass of *cis*-lycopene and *all trans*-lycopene were shown in Table 1 together with maximum wavelength of absorption spectrum of each. The level of lycopene extracted from *Citrullus lanatus* was 111 µg/g for *all trans*-lycopene and 19.9 µg/g for *cis*-lycopene. While the concentration extracted from *Lycopersiconesculentum* was 61.1 µg/g for *all trans*-lycopene and 37.4 µg/g for *cis*-lycopene. Reported from literature both watermelon and tomato juice was found to contain extractable lycopene of 20000µg/g, 18800 µg/g (94%) *all trans*-lycopene and 1200 µg/g (6%) *cis*-lycopene (Collins *et al*., 2004). It was also reported that in tomato the *all trans*- lycopene ranged from 79% to 91% while 9% to 21% was the *cis*-lycopene (Collins &Perkins-Veazie, 2006). Another literature reported that lycopene extracted from *Citrullus lanatus* contained 88% *alltrans*-lycopene and 12% *cis*-lycopene (Katherine, 2008).The results indicated that watermelon has higher content for both *all trans*-lycopene and *cis*-lycopene than tomato.

Table 1. Quantities of *all trans*-lycopene and *cis*-lycopene extracted from samples with their maximum wavelength of absorption spectra

Sample used	Mass of sample (g)	Lycopene isomers	Mass of lycopene isomer (g)		Mass of lycopene in µg/g of sample	Concentration of lycopene scanned in UV-VIS scan	Boiling point (^{0}C)	Maximum wavelength (λmax) of absorption spectrum characteristics before heating (nm)			Maximum wavelength (λmax) of absorption characteristics after heating at 74^{o}C for 2 hours (nm)			Maximum wavelength (λmax) of absorption spectrum characteristics after heating at 74^{o}C for 2 hours (nm)		
								Peak I	Peak II	Peak III	Peak I	Peak II	Peak III	Peak I	Peak II	Peak III
Citrullus lanatus	500	All trans	0.056	111	0.05	174	503	473	444	501 469	443	502	470 444			
		Cis-	0.009	19.9	0.02	172	502	471	444	500 469	444	502	470 444			
Lycopersicon esculentum	500	All trans	0.031	61.1	0.01	174	502	472	444	500 469	443	501	469 443			
		Cis-	0.019	37.4	0.01	172	502	471	444	500 469	443	501	469 443-444			

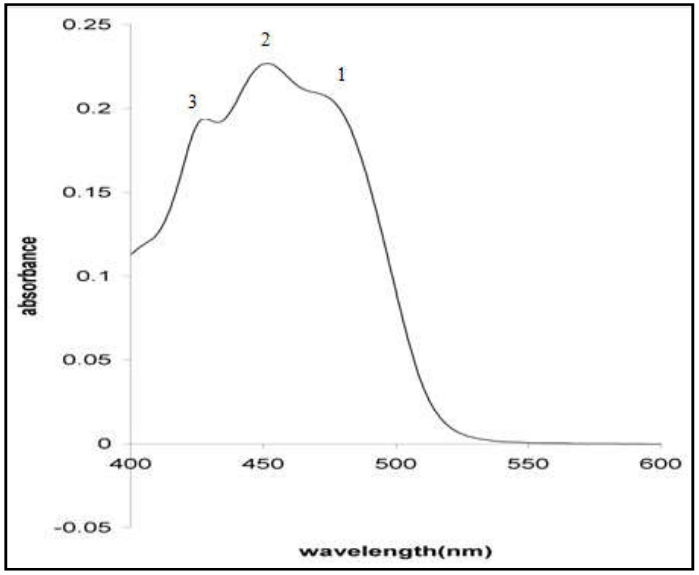

Figure 1. Absorption spectrum characteristics of beta carotene extracted from tomato. Beta carotene collected then scanned.
$\lambda_{1\ max}$ = 480 nm, $\lambda_{2\ max}$ = 451 nm and $\lambda_{3\ max}$ = 428 nm

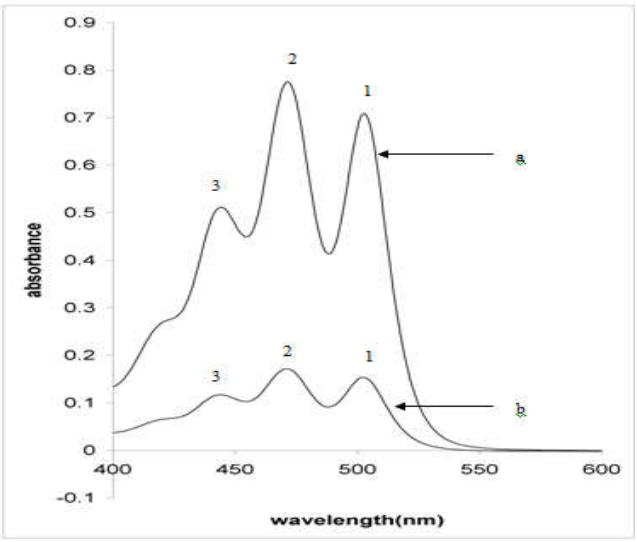

Figure 2. Normal derivative of absorption spectra characteristics of *all trans*-lycopene (b) and cis-lycopene (a) extracted from watermelon done before heating. Concentration of lycopene scanned in UV-VIS spectrophotometer was 0.015 g/litre in hexane solution

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

Other results are shown in Table 1

Also, from each sample, mass of *all trans*-lycopene is higher than mass of *cis*-lycopene extracted. Usually *cis*-lycopene occurs as trace in mixture of *all trans*-lycopene (Lunis, 2004).

Degradation of lycopene

The normal derivatives of absorption spectra characteristics of *all trans*-lycopene and *cis*-lycopene from watermelon has maximum wavelengths of (503, 473 and 444 nm) and (502, 471 and 444 nm) respectively (Figure 2). When heated at 74°C for 2 hours their maximum wavelengths were (501, 469 and 443 nm) for *all* trans-lycopene and (500, 469 and 444 nm) for *cis*-lycopene. When heated again at 74°C for 2 hours their maximum wavelengths were (502, 470 and 444 nm) for both *all trans*-lycopene and *cis*-lycopene, indicating that both two compounds exists as cis-lycopene. The normal derivatives of absorption spectra characteristics of *all trans*-lycopene and *cis*-lycopene from tomato had maximum wavelengths of (502, 472 and 444 nm) and (502, 471 and 444 nm) respectively. When heated at 74°C for 2 hours their maximum wavelengths were (500, 469 and 443 nm) for both *all trans*-lycopene and *cis*-lycopene. Both compounds from tomato have been degraded completely to cis-lycopene. The significance of this result is that *all trans*-lycopene from watermelon takes longer time to be converted to *cis*-lycopene by heating rather than *all trans*-lycopene from tomato and hence gives a preference of watermelon for dietary purpose to tomato. From literature, the result is showing that spectral characteristics of *all trans*-lycopene has maximum wavelengths of (500, 470 and 442 nm) (Genival *et al.,* 2008). Spectral characteristics of 13-*cis*-lycopene from freezed-dried pitanga pulp has maximum wavelengths of (496, 466, 441 and 360nm) (Genival *et al.,* 2008). Another result is showing that the maximum absorption spectra characteristics of lycopene has maximum wavelength of (502, 470 and 444 nm) in petroleum ether (Rodriguez-Amaya, 2004). As in petroleum ether the absorption peaks of lycopene in hexane is (444, 470 and 502 nm) (Wrolstad, 2005).

The melting point of lycopene

The measured melting points of *all trans*-lycopene and *cis*-lycopene extracted was as follows:

All trans-lycopene of both tomato and watermelon = 174^{0}C
Cis-lycopene of both tomato and watermelon = 172^{0}C

Comparing with literature the melting point of lycopene is 172°C –175^{0}C (Gerster, 1997). The melting point of *all trans*-lycopene is 172^{0}C – 173^{0}C (Rosenberg, 1961). Melting point of *cis*-lycopene is 172^{0}C – 175^{0}C (Sengupta & Das, 1999). The result of this research is quiteclose 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.

Mass of *cis*-lycopene and *all trans*-lycopene together with maximum wavelength of absorption spectrum of each were shown in the following Table 1:

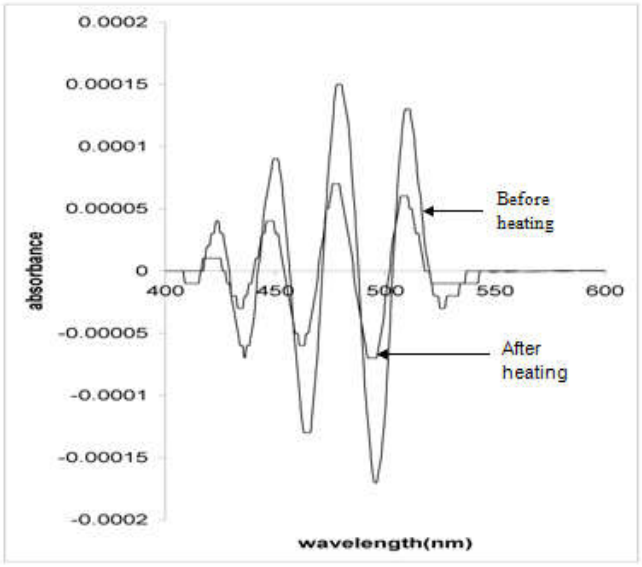

Figure 3. 2nd derivatives of absorption spectra characteristics of *all trans*-lycopene extracted from tomato before and after heating at 74^{0}C temperature for 2 hours in hexane solution. Concentration of lycopene scanned in UV-VIS spectrophotometer was 0.01 g/litre in hexane solution

Conclusion

Both watermelon and tomato gives the same trend of high quantity of *all trans*- lycopene compared to *cis*-lycopene, however watermelon gives higher content of all trans-lycopene than tomato. The time taken (4 hrs) for *all trans*-lycopene and *cis*-lycopene from watermelon to be heated (at 74^{0}C) so that their maximum wavelengths of absorption spectra characteristics to be equal is longer than time taken (2 hrs) for *all trans*-lycopene and *cis*-lycopene from tomato to be heated at the same temperature. Thus lycopene from watermelon is more stable to undergo degradation by heat than lycopene from

tomato. When *all trans*-lycopene from watermelon and tomato were heated there was a change for maximum wavelength of absorption spectra. When scanned in UV-VIS spectroscopy the second derivatives of their characteristics show the differences from the original characteristics (before heating), an example is shown in Figure 3 above.

Recommendation

Conditions for minimum degradation of lycopene should be considered during food processing of tomato products and other products. It is far better to repeat the procedure for about four extra sets of samples of both tomato and watermelon.

Acknowledgement

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

REFERENCES

Basu, A. and Lmrhan, V. 2007. Tomato versus lycopene in oxidative stress and carcinogenesis: Conclusions from clinical trials. *European Journal of Clinical Nutrition*, 61(3), 295 - 303.

Caris-Veyrat, C. and Genard, M. 2008. How does a tomato quality (sugar, acid and nutritional quality) vary with ripening stage, temperature and irradiance. *Journal of Agricultural Food Chemistry*, 56(4), 1241 - 1250.

Collins, J. K. and Perkins-Veazie, P. 2006. Lycopene: From Plants to Humans. *HortScience*, 41(5), 1135-1144.

Collins, J.K.; Perkins-Viazic, P. and Robert, B.W. 2004. A new World of Watermelon. Agricultural Research Magazine, (December),10 - 11**.**

Genival, L. F., A; De Rosso, V.V. ; Angela, M.; Meireles, A.; Rosa, P.T.V.; Oliveira, A.L. ; Mercadante, A.Z.; Cabral, F.A. 2008. Supercritical CO2 extraction of carotenoids from pitanga fruits (*Eugenia uniflora* L.). *Journal of Supercritical Fluids*, 46, 33 – 39.

Gerster, H. 1997. The potential role of lycopene for human health. *Journal of American Coll Nutrition*, 16(2), 109 - 126.

Katherine, L. V.2008. Extracted Conducting Affecting Supercritical Fluid Extraction of Lycopene from Watermelon. *Journal of Agricultural Food Chemistry*, 99(16), 7835 – 7841.

Kun, Y.; Lule, U. S. and Lin, D. X. 2006. Lycopene: Its Properties and Relationship to Human Health. *Journal of Food Review International*, 22**(**4**)**, 309-333.

Lunis, N.H. 2004. Production of Lycopene Crystal From Tomato Waste. *Journal of Science and Technology*, 24(3), 440 – 447.

Morris, A.; Cunnighai, S.; Benos, D. and Frizzell, R. 1994. Process for the isolation and purification of lycopene crystal. *Journal of American Association*, 272(18), 1439 - 1441.

Rodriguez-Amaya, D.B. 2004. HarvestPlus Handbook for Carotenoid Analysis.

Rosenberg, B. 1961. Photoconduction Activation Energies in Cis-Trans Isomers of Beta-Carotene *Journal of Chemical Physics,* 34(1), 1 - 4.

Sengupta, A. and Das, S. 1999. The Anti-Carcinogic Role of Lycopene, Abundently Present in Tomato. *European Journal of Cancer,* 8(4), 325 – 30.

Sesso, HD and Liu, S; Gaziano, J.M; Buring, J.E. 2003. Dietary lycopene, tomato-based food products and cardiovascular disease in women.*J Nutr.;* 133(7):2336 - 41.

Shi, J. and Maguer, M. 2000. Lycopene in Tomatoes: Chemical and Physical Properties Affected by Food Processing. *Journal of Critical Reviews in Food Science and Nutrition*, 40, 1 - 42.

Tan, B. 2006. Analytical and Preparative Chromatography of Tomato Paste Carotenoids*. Journal Food Science,* 53(3), 954 - 959.

Wrolstad, R.E. 2005. F.2.2. Detectioon and Measurement of Carotenoid by UV/VIS Spectrophotometer. Handbook of Food analytical Chemistry, Volume 1: water, protein, enzymes, lipids and carbohydrates.

Appendix

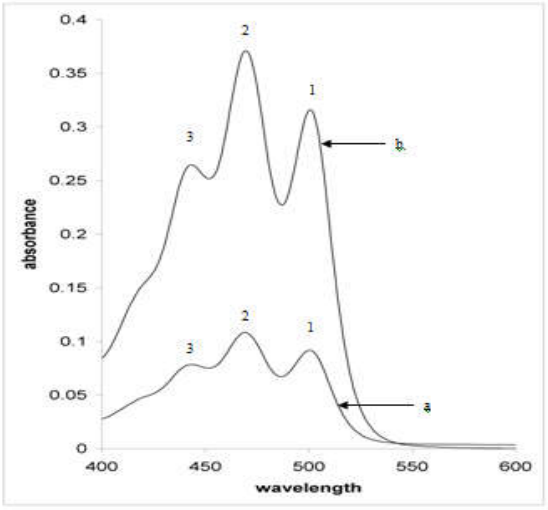

Figure 4. Normal derivative of absorption spectra characteristics of all trans- (b) and cis- lycopene (a) extracted from watermelon and scanned in UV-VIS spectrophotometer with concentration of 0.015 g/litre in hexane solution after heating for 2 hours at 74^{0}C temperature

In b $\lambda_{1\ max}$ = 501nm, $\lambda_{2\ max}$ = 469nm and $\lambda_{3\ max}$ = 443nm.
In a $\lambda_{1\ max}$ = 500nm, $\lambda_{2\ max}$ = 469nm and $\lambda_{3\ max}$ = 444nm

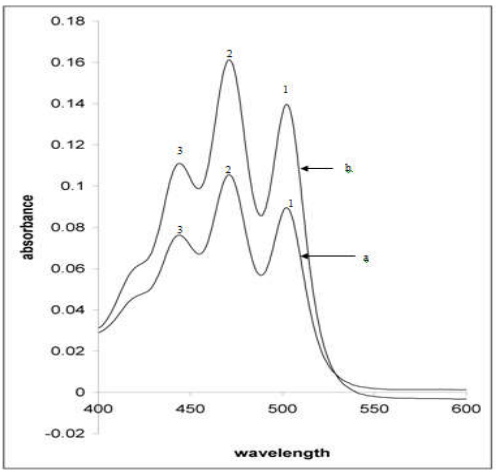

Figure 5. Normal derivative of absorption spectra characteristics of all trans- (b) and cis- lycopene(a) extracted from tomato before heating in hexane solution. Concentration of lycopene scanned in UV-VIS spectrophotometer was 0.01 g/litre in hexane solution for each of the two

In b $\lambda_{1\ max}$ = 502nm, $\lambda_{2\ max}$ = 472nm and $\lambda_{3\ max}$ = 444nm
In a $\lambda_{1\ max\ I}$= 502nm, $\lambda_{2\ max}$ = 471nm and $\lambda_{3\ max}$ = 444nm

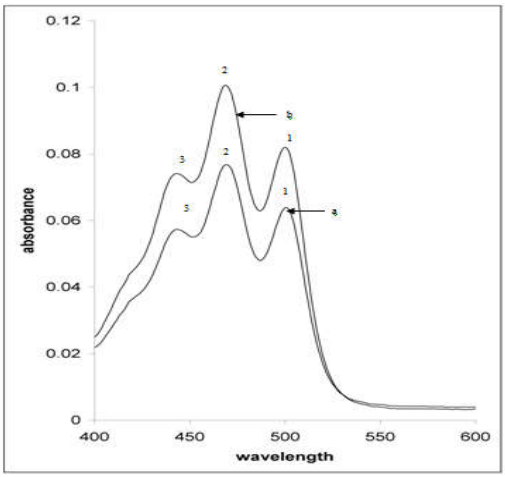

Figure 6. Normal derivative of absorption spectra characteristics of all trans-(b) and cis- lycopene(a) extracted from tomato after heating at 74^{0}C temperature for 2 hours in hexane solution. Concentration of lycopene scanned in UV-VIS spectrophotometer was 0.01 g/litre in hexane solution

In b $\lambda_{1\ max}$ = 500nm, $\lambda_{2\ max}$ = 469nm and $\lambda_{3\ max}$ = 443nm
In a $\lambda_{1\ max}$ = 500nm, $\lambda_{2\ max}$ = 469nm and $\lambda_{3\ max}$ = 443nm

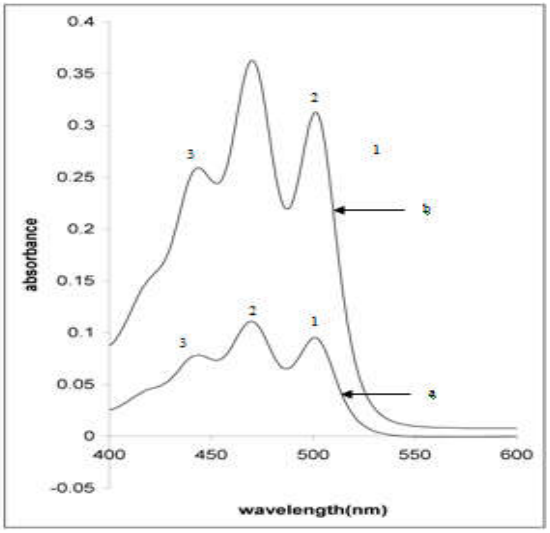

Figure 7. Normal derivative of absorption spectra characteristics of all trans-(b) and cis- lycopene (a) extracted from watermelon after heating at 74^{0}C temperature for 2 hours in hexane solution

In b $\lambda_{1\ max}$ = 502nm, $\lambda_{2\ max}$ = 470nm and $\lambda_{3\ max}$ = 444nm
In a $\lambda_{1\ max}$ = 502nm, $\lambda_{2\ max}$ = 470nm and $\lambda_{3\ max}$ = 444nm

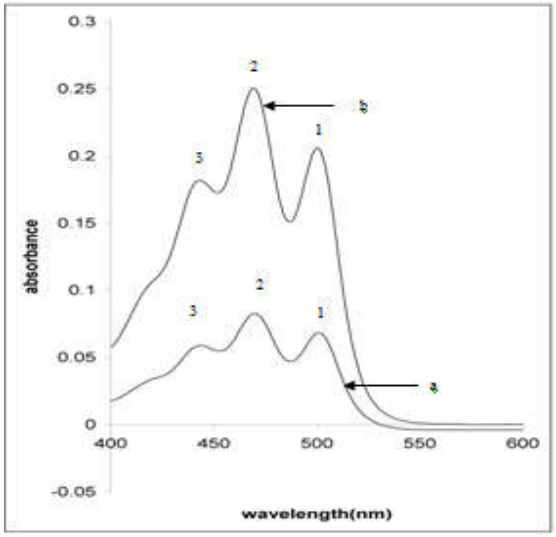

Figure 8. Normal derivative of absorption spectra characteristics of all trans-(b) and cis-lycopene (a) extracted from tomato after heating again at 74^0C temperature for 2 hours in hexane solution

In b $\lambda_{1\ max}$ = 500nm, $\lambda_{2\ max}$ = 469nm and $\lambda_{3\ max}$ = 443nm
In a $\lambda_{1\ max}$ = 501nm, $\lambda_{2\ max}$ = 469nm and $\lambda_{3\ max}$ = 443-444nm

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