International Journal of Advanced Engineering and Management Research
Vol. 3 Issue 5; 2018

www.ijaemr.com ISSN: 2456-3676

PURITY DETERMINATION OF LYCOPENE EXTRACTED FROM SELECTED TOMATO AND WATERMELON VARIETIES

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Abstract

Tomatoes (Solanum lycopersicum) and red-fleshed watermelons (Citrullus lanatus) contain lycopene, which have many important health benefits. However, no study has so far been done to establish the degree of purity of lycopene in fresh tomatoes and watermelons in Uganda, and East Africa in general. The objective of this study was to separate and purify lycopene in selected tomato and watermelon varieties in Uganda and then determine its % purity. 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 (extracts) were then separated from the filtrate by using separating funnel, washed, dried by rotary evaporator and then dissolved in hexane. The concentrated hexane solution was then fractionated by using the alumina column chromatography. The purity of lycopene was determined by using high performance liquid chromatography (HPLC). The % purity of lycopene was calculated by dividing the area of lycopene peak to the total area of peaks from the HPLC chromatogram. The results obtained showed that the quantity of lycopene varied from 140 µg/g to 791 µg/g wet weight in tomato and for watermelon was 326 µg/g. The % purity ranged from 76.3% to 87.8% for tomato varieties. The chromatograms of lycopene from watermelon did not appear properly. Purity products are committed to providing customers with evidence based nutrition's supplements. The % purity of extracted lycopene may be increased by crystallization of lycopene.

Keywords: Solanum lycopersicum, Citrullus lanatus, lycopene, purity, percentage, chromatography, nutrition.

Introduction

Lycopene is a pigment which is principally responsible for the characteristic deep-red colour of ripe tomato fruits and tomato products (Shi, 2000). It occurs naturally in tomato as carotenoid and is a major component found in the serum of tomato (Kun et al., 2006). Other significant sources are Red-fleshed watermelon (such as Citrullus lanatus), pink grapefruit, pink guava, papaya and apricots (Gerster, 1997). Lycopene is a C40-carotenoid made up of eight isoprene units. Its molecular weight is 536.9 (Gerster, 1997). Dietary lycopene has ability 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, 2000). Development of lycopene-rich food, as well as food-grade and pharmaceutical-grade lycopene is needed by consumer from the food industry of high quality lycopene products that meets food safety regulations (Shi, 2000). It is important to know the degree of purity of lycopene before being fortified in any food or commercial food products. Lycopene standard with high degree of purity is also needed for laboratory and industrial purposes. The objective of this study was to determine the degree of purity of lycopene after extracted from watermelon and tomato varieties.

Materials and Methods

Apparatus

Beakers, conical flasks, measuring cylinders, droppers, rotary evaporator, glass column separation for chromatographic separation, analytical balance, nitrogen gas (for drying lycopene extracts) and Ultra Violet - Visible radiation Spectrophotometer for scanning lycopene samples. Then, C18 HPLC column: length =250 mm, RP-18(7µm) i.e. particle size = 7 µm, internal diameter = 4.6 mm, Hibar repacked column, manufacturer: Cica-Merck, serial number: 61264 was used. High Performance Liquid Chromatography (HPLC) with UV-VIS detector.

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, trietthyl amine and tetrahydrofuran.

Sample Collection

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

Tomato samples

Elliptical tomatoes (Lycopersicon esculent), small spherical tomatoes (Solanum quitoense) and large spherical tomatoes (Sloane lycopersicum cerasiforme) varieties.

Watermelon (Catullus landaus) samples

Red-fleshed watermelon 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 30g and 215g 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 samples of 30 g and 215 g of grounded tomato were each put in a beaker and extracted with a mixture of ethyl acetate, acetone and hexane (1:2:4 v/v/v) 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 or stream of nitrogen without heating (Tan, 2006). A sample of 215 g of flesh of watermelon was also grounded, extracted using the same solvent mixture, repeated once, mixed the extracts and then concentrated (Collins et al, 2004).

Purification by washing

Before a chromatographic separation, the lycopene-containing organic layer was separated from two layers of original extract by using funnel separation. The lycopene-containing organic layer was then washed by using saturated sodium chloride solution, followed by 10% aqueous potassium carbonate and another portion of saturated sodium chloride solution. The lycopene containing organic layer was then dried with anhydrous magnesium sulphate (Hauptmann, 2004).

Preparation of a Column

A chromatography column was mounted at a retort stand vertically. A small amount of cotton was placed at the bottom of column followed by hexane (5 ml). Clean sand (1 cm) was added to the column. Then, activated alumina (13g -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).

Column Chromatographic Separation of Lycopene from Lycopene Extract

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. The extracts were dried and then weighed.

The melting point of lycopene was measured and recorded 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.

Purity determination of Lycopene by HPLC

Dissolution of the dry extract in tetrahydro furan / acetonitryl /methanol (15:30:55 v/v/v) was done. The solution was injected on a C18 column with methanol/acetonitryl (90:10 v/v) and triethyl amine (9 mL in volume) as mobile phase (Φ = 1.3 ml/min) and λdetection = 475 nm (Barba et al, 2006). The lycopene was dissolved in 5 ml of the solvent system of tetrahydrofuran/acetonitrile/methanol (15/30/55: v/v/v). 20 µL of the prepared solution was injected to the HPLC column and allowed to flow at a rate of 0.9 ml/min for the first injection and 1.3 ml/min for other injections so as to increase the rate flow for 30 min, with mobile phase of methanol/acetonitrile (90:10 v/v) mixed with 20 mL of triethyl amine.

Results and Discussion

Purity of Lycopene Extracted

The % purity of lycopene was determined from calculation by dividing the area of lycopene peak to the total area of arytenoids peaks from the HPLC chromatogram. The % purity of lycopene

extracted from Lycopersicon esculent was 80.8, from Sloane lycopersicum cerasiforme was 87.8 and from Solanum quitoense was 76.3, as list in Table 1.

From literature lycopene extracted from tomato waste was 98% purity (Navigilo et al, 2008). Also purity of lycopene was increased from 96.4% to 98.1% (Nunes, 2007).

Comparing with the results of literature the % purity of lycopene from literature was higher than the % purity of lycopene obtained from this study.

The Melting Point of Lycopene

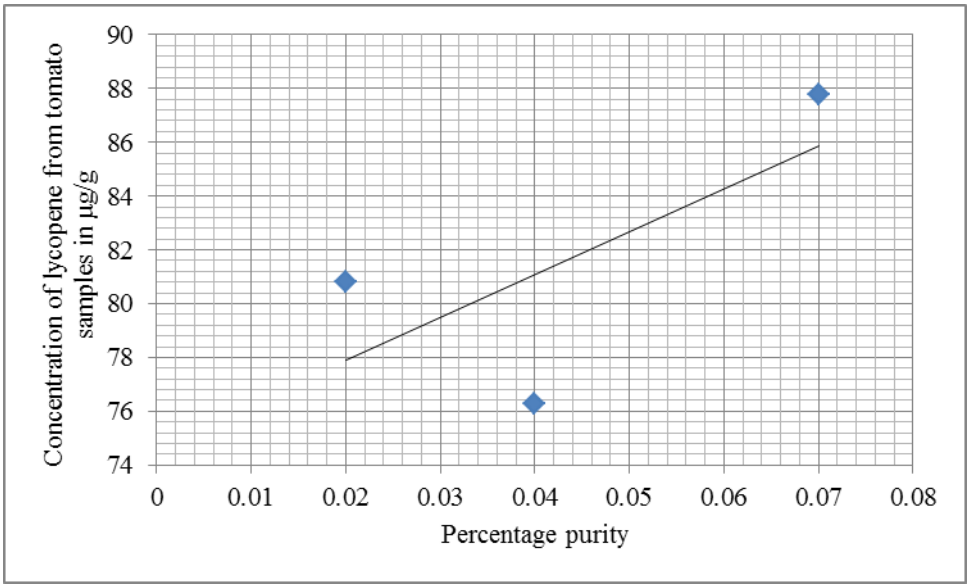
The melting point of lycopene (with purity determination) extracted from small spherical tomato and watermelon was found to be 1700C -1720C. From literature the melting point of lycopene is 1720C - 1730C (Greater, 1997). The result is quite close to that in literature and the short deviation might be 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.

The % purity of Catullus landaus was not calculated because its chromatogram did not appear well.

Table 1: Quantities of Lycopene Extracted from Samples with their %Purity

Sample S/N		Mass of sample in g	Mass of lycopene in g	Mass of lycopene in µg/g of sample	Concentration (g/litre) of lycopene scanned in UV-VIS Scan	% purity
Citreous	**1**	215	0.07	326	0.020	
Lantos						
Lycopersicon esculent	**1**	215	0.07	279	0.020	80.8
Sloane	**1**	215	0.03	140	0.07	87.8
lycopersicum cerasiforme						
Solanum quitoense	**1**	215	0.17	791	0.04	76.3
	2	30	0.01	333	0.167	

The following Figure 1 shows the correlation of concentration of lycopene extracted from tomato samples and percentage purities of those samples:



Correlation = 0.691

Figure 1: correlation of concentrations of lycopene extracted from tomato varieties and the percentage purities of lycopene extracted

In Figure 1 above the percentage purity of lycopene extracted from tomato varieties increased with the increase in concentration of lycopene.

The followings, Figure 2, Figure 3, and Figure 4, showing the chromatograms of lycopene extracted from tomato varieties:

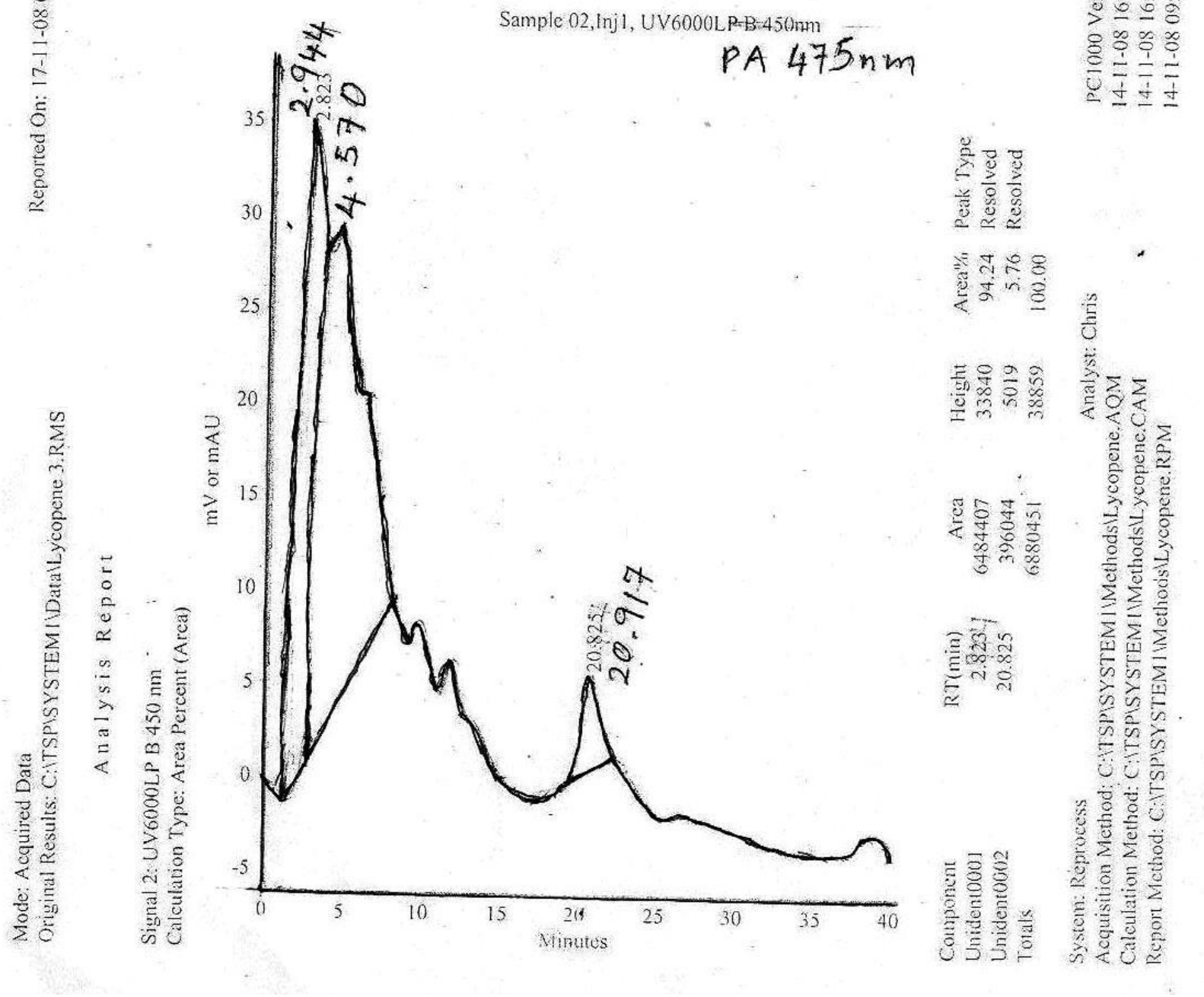


Figure 2: Sample 02 Chromatogram of Lycopene Extracted from Large Spherical Tomato (Lycopersicon Esculentum)

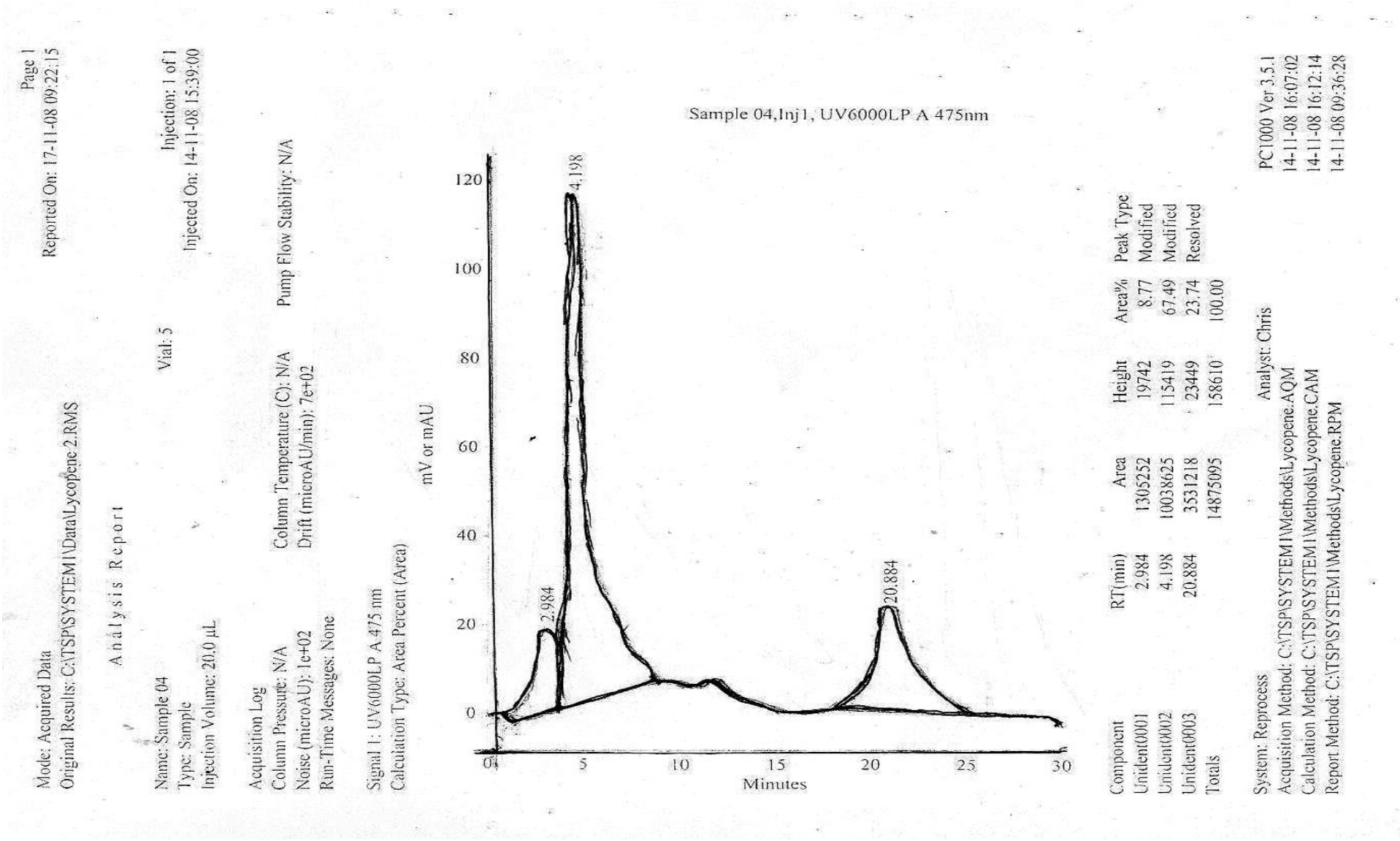


Figure 3: Sample 04 Chromatogram of Lycopene Extracted from small Spherical Tomato (Solanum quitoense)

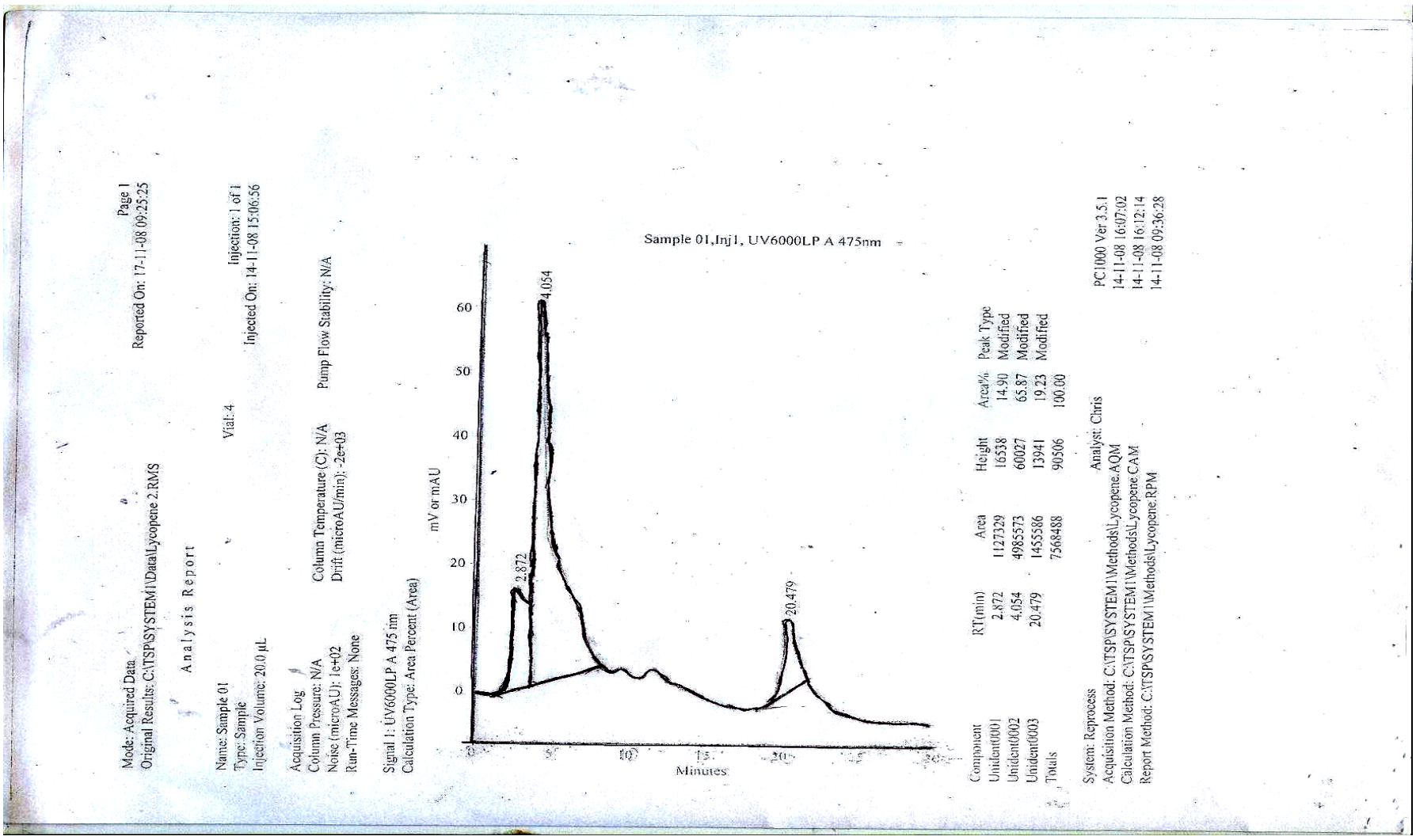


Figure 4: Sample 01 Chromatogram of Lycopene Extracted from Elliptical Tomato *(Sloane lycopersicum cerasiforme)*

Figure 5 below shows the variation of percentage purity of lycopene extracted from tomato varieties

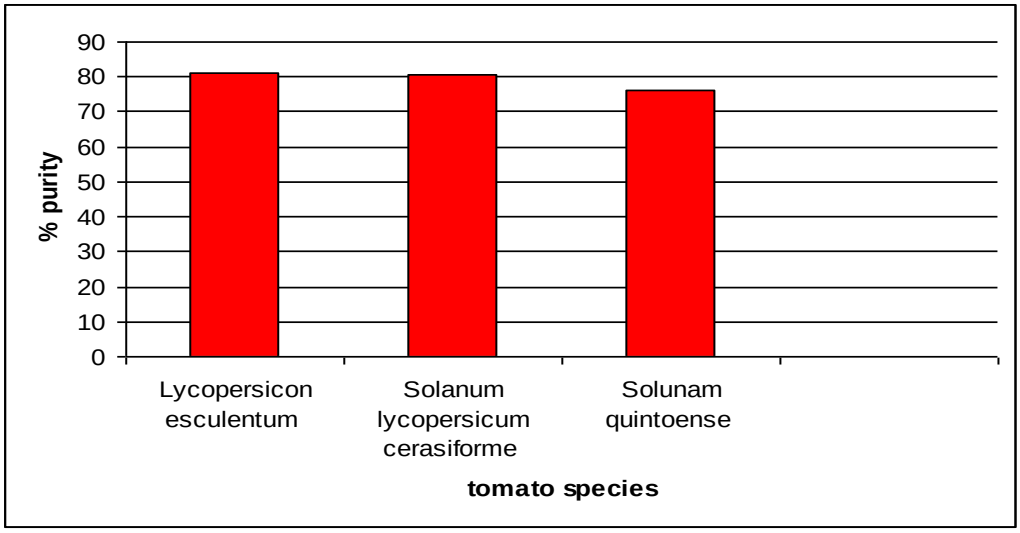


Figure 5: Variation of Purity of Extractable Lycopene with Species in Tomato

The following Figure 6 shows the chromatogram of pitang plant extract:

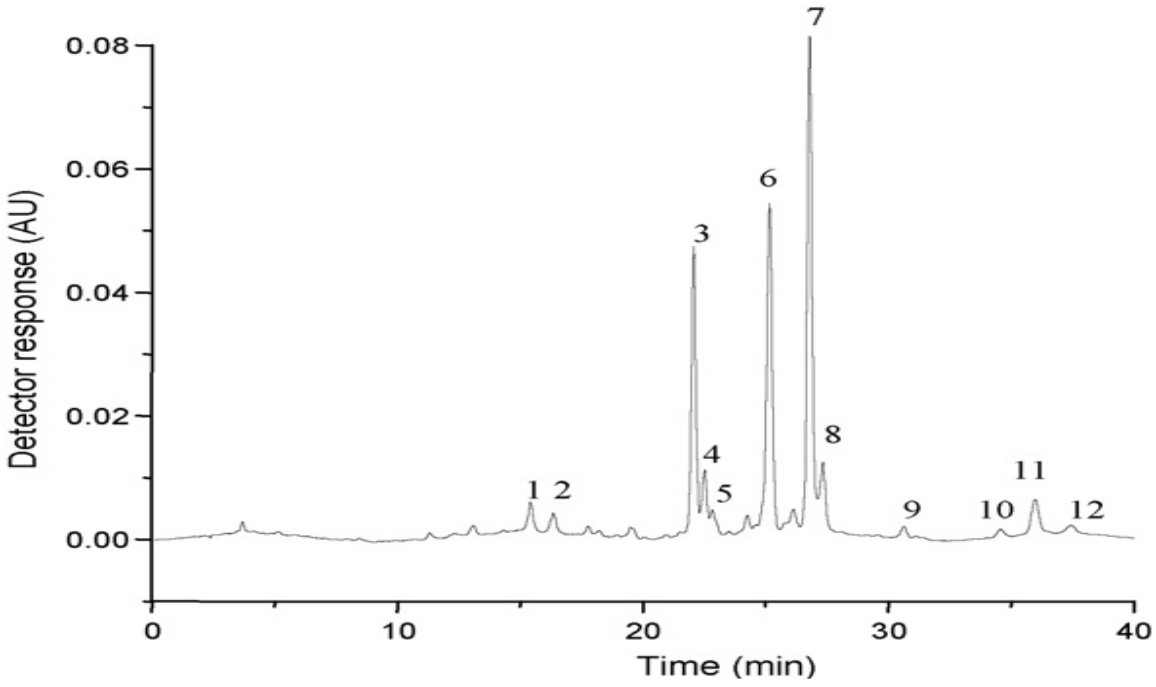


Figure 6: HPLC–PDA Chromatogram of the Freeze-Dried Pitanga Extract Extracted by Acetone: (1) all-trans-lutein, (2) all-trans-zeaxanthin, (3) all-trans-rubixanthin, (4) cis-rubixanthin I, (5) cis-rubixanthin II, (6) all-trans- cryptoxanthin, (7) all-trans lycopene, (8) 13-cis-lycopene, (9) all-trans-γ- carotene, (10) all-trans-α-carotene, (11) all-trans-carotene + 9-cis-carotene and (12) 15-cis-carotene + 13-cis- carotene (Rodriguez-Amaya, 2004).

Conclusion

The results show that the quantity and % purity of extracted lycopene from tomatoes varied. Purity products are committed to providing customers with evidence based nutrition's supplements.

Recommendation

The % purity of extracted lycopene may be increased by recrystallization of lycopene. Lycopene with high degree of purity is very important for laboratory and industrial works as lycopene standard and ingredients in industrial food products, pharmaceutical syrupy and drips.

Acknowledgement

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

Reference

Barba, A.I.O.; Hurt ado, M.C.; Sanchez-Mata, M.C.; Ruiz, V.F. and Tejada, M.L. (2006). Analytical, Nutritional and Clinical Methods: Application of a UV–VIS detection-HPLC method for a rapid determination of lycopene and β-carotene in vegetables. Journal of Food Chemistry, 95(2), 328-336.

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

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

Hauptmann, R. (2004). Process for Isolation and Purification of Lycopene Crystals. Online (2004), available from https://patents.google.com , (excess 01-01-2004).

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.

Navigilo, D.; Pizzolongo, F.; Navigilo, B.; Aragon, A. and Santini, A. (2008). Extraction of Pure Lycopene From Industrial Tomato Waste in Water Using the Extractor Navigilo. Journal of Food Science, 2(4), 0337 – 0344.

Nunes, H.L. (2007). Production of Lycopene Crystal from Tomato Waste. Journal of Science and Technology, 24(3), 440 – 447.

Rodriguez-Anaya, D.B. (2004). Harvest Plus Handbook for Carotenoid Analysis.

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.