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**OPTIMIZATION OF LYCOPENE FROM MALAYSIAN ALL SEASON COLOURED
FLESHY FRUITS**

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ABSTRACT

Lycopene is a carotenoid that has antioxidant properties and imparts the red pigment in some fleshy fruits and vegetables. Mango (*Mangifera indica* L), papaya (*Carica papaya*), Black berry, Water-melon and Tomato (*Lycopersicon esculentum* Mill.) are famous coloured flesh all seasonal fruits in Kelantan (Malaysia). These fruits also one of the predominant lycopene sources in a typical Malaysian diet. It has attracted attention due to its biological and physicochemical properties, especially related to its effects as a natural antioxidant. Since lycopene has health benefits and food colorant potential, lycopene content fruits are of interest to the food industry. Lycopene, not soluble in water, was recovered in a powder-crystalline solid form and purified by solid phase extraction using a small amount of organic solvent. The all trans lycopene was obtained at a very high grade of purity, not less than 96% (w/w), with an average recovery from 8-12% (w/w) from all coloured fleshy fruits in Malaysia. As it is clear, natural products are good sources of antioxidants and evaluation of economic costs and simplicity and availability of extraction methods are very necessary. So in this present works, economic extraction and quantification method of lycopene from Malaysian all seasonal fleshy fruits have been investigated.

Keywords: Coloured Fleshy Fruits, Lycopene, Carotenoid, Lycopene Assay

INTRODUCTION

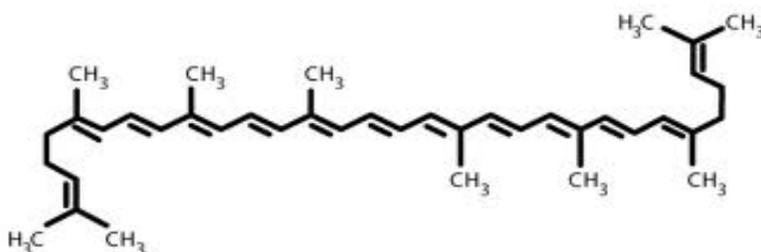
Lycopene, a fat soluble carotenoid, is a precursor of β -carotene and has at least twice the antioxidant capacity of β -carotene [1]. Good amounts of lycopene are contained in many natural products, like ripe mango, tomato (*Lycopersicon esculentum* Mill), water-melon, red pepper, black berry, papaya etc. [2]. Lycopene, which is one of the main carotenoids in tomatoes, varies with different ripening stages, and is mainly responsible for the red colour [3]. In the last few years the importance of lycopene rapidly increased due to its pharmacological and anti carcinogenic properties [4, 5]. Epidemiological studies indicate a protective effect of lycopene against some types of cancer, e.g., stomach and prostate cancer [6], so the demand for this molecule and for the carotenoids in general, is rapidly growing [7]. Recent epidemiological studies have suggested that the consumption of tomatoes and tomato-based food products reduce the risk of cancer (oral cavity, pharynx, esophagus, stomach, rectum, colon, urinary bladder, prostate and breast) in humans [8-10]. It has attracted substantial interest during recent time for its beneficial in reducing oxidative stressing coronary heart diseases and other chronic diseases [11-14].

The pharmacokinetic properties of lycopene remain particularly poorly understood. An industrial scale, environmentally friendly lycopene extraction and purification procedure with minimal loss of bioactivities is highly desirable for the foods, feed, cosmetic, and pharmaceutical industries. High-quality lycopene products that meet food safety regulations will offer potential benefits to the food industry. In the literature, different synthetic pathways for this molecule have been reported, but in all cases the synthesis of lycopene seems to be very expensive and economically not convenient procedure [15]. Therefore, an easy, inexpensive, and reliable method to determine lycopene content over a broad spectrum of fruits and varieties that does not require hazardous chemicals is needed for the food industry.

The northern parts of Malaysia (Jeli, Kelantan) is blessed with the richest delicious all seasonal coloured fruits flora on the earth. The most common fruits are being different types of mango, Papaya, water melon, black berry, tomato etc. When these fruits mature & rip, it shows yellow to red colour. The colour change in tomatoes is a combination of processes including the breakdown of chlorophyll and build-up of carotenes [16]. These colour fruits supply maximum amount of lycopene

to our diet. But due to lack of storage facilities and proper transport system around 30-40% of the total country cultivation fruits spoilage. So this wastes fruits products may be used as an ingredient in animal food, could be more efficiently used as a source of pure lycopene. Considering both the availability of the wastes mature

fruits at the optimum fruits season, the relevant interest for this molecule and its biological activity, the main aim of this work is to propose a new method for the extraction and characterization of lycopene from different Malaysian coloured wastes fleshy fruits.



Lycopene; Molecular Weight = 536.89; Exact Mass = 536; Molecular Formula = C₄₀H₅₆

MATERIALS AND METHOD

Materials

Lycopene standard was purchase from (Fluka, Japan). High-performance liquid chromatography (HPLC) solvents were purchased from Fisher Scientific (USA). All reagents were of analytical grade (UK, Japan and China). Fresh ripe tomatoes, mango, watermelon, papaya, blackberry were purchased from a local market (Jeli Bandar, Kelantan) and stored at 4°C for a maximum of 2 days before use. Acetone, ethanol, ethyl acetate and hexane were obtained from Fluka, Japan. Their purities were greater than 99.7%, 99.5%, 99% and 95%, respectively.

Sample Preparation (Mango, Black berry, Water-melon, Papaya)

Good ripe tomatoes, mango, papaya, water-melon, and black berry were purchased from the open fruits market at Jeli Bandar, Kelantan, so variety names were not available. All the experimental fruits flesh was fully mature and colour. After collecting the fruits, it was washed with water under a tap and store in a bamboo basket finally dried on a table by wind using fan. Then by wearing hand gloves the fruit coloured tissue was cut into small pieces with sharp knives. Fresh tomato, mango, papaya, water-melon, black berry only coloured parts of the fruits flesh samples (500 g) were homogenized in a electric blender until chunks were 4-3mm. Fresh coloured fruits flesh were diluted 1:1 (W:V) in deionised water before blending. All

fruits flesh samples were pureed using an Electric Homogenizer (Jem, UK) with a 20 mm O.D. blade to produce uniform slurry

with particles smaller than 2 mm. The samples were not allowed to heat or froth. Puree content around (16%) moisture.



Picture of Coloured Malaysian Fleshy Fruits

Low Volume Hexane Extraction Method

The low volume hexane extraction method (LVHEM) was performed as in [17]. Approximately 1g duplicate samples were weighed from each puree into two 40 ml amber screw-top vials (Fisher) that contained 5 ml of 0.05% (w/v) butylated hydroxytoluene (BHT) in acetone, 5 ml of 95% ethanol, and 10 ml of hexane. Purees were stirred on a magnetic stirring plate during sampling. Samples were extracted on an orbital shaker at 180 RPM for 15 min on ice. After shaking, 3 ml of deionized water were added to each vial and the samples were shaken for an additional 5 min on ice. The vials were then left at room temperature for 5 min to allow for phase separation. The hexane phase was removed and retained. The process of hexane addition, mixing, and centrifugation was repeated three times. Extracts were pooled and dried in a Rota-evaporator (model 160; England), and stored in a -20°C freezer. The whole process was performed under yellow light. The absorbance of the upper, hexane layer

was measured in a 1 cm path length quartz cuvette at 503 nm blanked with hexane. The lycopene content of each sample was then estimated using the absorbance at 503 nm and the sample weight [17].

Calculation of Lycopene Levels

Lycopene levels in the hexane extracts were calculated according to:

$$\text{Lycopene (mg/kg fresh wt)} = \frac{A_{503} \times 537 \times 8 \times 0.55}{0.10 \times 172} \quad \text{---- (1)}$$

$$= A_{503} \times 137.4 \quad \text{----- (2)}$$

where 537 g/mole is the molecular weight of lycopene, 8 ml is the volume of mixed solvent, 0.55 is the volume ratio of the upper layer to the mixed solvents, 0.10 g is the weight of coloured puree added, and 172 mM^{-1} is the extinction coefficient for lycopene in hexane.

Study of The Influence of Extraction Conditions on Yields

The greatest improvement in lycopene extraction was used to investigate the effects of solvent type, blending time and number of extraction on yields. Hexane, ethyl acetate or the ternary mixture hexane/acetone/ethanol (50:25:25 v/v) were

used as solvent. The latter was chosen because of its proven efficacy for the extraction of carotenoids from plant material [18]. The blending time was varied between 2 and 5 minutes. Optimum time is 3 minutes. Lycopene extraction was carried out according to the procedure described in the previous section. These values were found to be a good compromise between the efficiency of extraction and the loss of lycopene due to oxidation.

RESULTS AND DISCUSSION

In order to identify the lycopene, a few crystal of extracted lycopene was dissolved in concentrated sulphuric acid, imparting an indigo blue colour to the solution. In another test, by adding a solution of antimony trichloride in chloroform to a solution of lycopene in chloroform, an intense unstable blue colour appeared. Our extracted all sources lycopene compound gives the positive test with the above reagents. These tests primarily proved the presence of lycopene in the extract. UV Spectrum is shown in **Figure 2**, the maximum wave lengths are 447.2, 473.2 and 504.2 nm, which is the maximum wavelengths of pure lycopene reported in the literatures.

Extraction yields were expressed as mg of lycopene per 100 g of dry plant material and mg/kg for fresh weight fruits. Depending on the material considered, the moisture content was between 85 and 95 wt % (**Table**

1), while the total lycopene content was between 310 and 460 mg/100 g dw and 50-75 mg/kg (water melon) fresh weight (**Table 2**). Differences in the lycopene content for the mango, tomato, papaya, water melon, black berries may be due to the different ripeness of the fruits used and/or the storage conditions of the material and also major condition is that, different species and varieties of fruits.

We tested the reduced volume method could fulfil two fundamentals requirements i.e. lycopene extracted by the medium and the hexane phase obey Beer's-Lambert law under this assay protocol. We prepare fresh watermelon tissue puree and added various quantities of the puree for reduced volumes of extraction. To test that reduced volume protocol extracted all of the lycopene in the samples, the aqueous phase of each was re-extracted with a second 10ml of hexane and the absorbance at 503nm determined.

The absorbances of the second hexane extracts ranged from < 0.002 for the 0.5g of tissue to < 0.02 for 2.0 gm of tissue. These results shows that all lycopene be released from the tissue and extracted into the hexane phase. The average lycopene content in watermelon ranged between 40-75mg/kg which was supported by previously reported [19, 20].

Study of The Influence of Extraction Conditions on Yields

For all investigated solvents, maximum lycopene extraction yields in hexane, ethyl acetate and the mixture hexane / acetone / ethanol (50:25:25) were 346.4, 334.3 and 460.2 mg/100gdw, respectively. Increasing the blending time resulted in a progressive reduction in yields. This suggests that the enzymatic degradation of cell-wall components is very fast and occurs within the 4 to 5 minutes blending. As regards the influence of solvent type, we note that using hexane or ethyl acetate did not produce significant differences in extraction efficiency. In contrast, the mixture hexane/acetone/ethanol (50:25:25) appeared to be much more effective. We also studied about numbers of extraction with the different solvent systems. Lycopene extracted 60-70% by the first extraction and 10-25% by 2nd extraction. So, concluded that two times extraction with lycopene extracted solvent is suitable for 90-95% of total lycopene present in fruits materials.

Hexane was used as the extraction solvent in this study. Although solvent mixtures that contain hexane, acetone, and ethanol have often been used in different studies and have been suggested to be of higher extraction efficiency [21], these mixtures also require a longer time to evaporate the solvent mixture than hexane. Lycopene yield increased as

the volume of hexane and the period of vortex increased and similar trends were also observed in other studies [22, 23]. It is noteworthy that Periago and coworkers [21, 22] also evaluated optimizing lycopene extraction from tomato and tomato products, although they did not use the response surface methodology. Since hexane is the only component of the mixture with a high affinity for lycopene, it follows that acetone and ethanol must play some auxiliary role in the overall extraction process. A possible explanation is that the two polar compounds, due to their small molar volume, high hydrogen bonding capability and large basicity, could cause the swelling of the plant tissue, thus facilitating solvent penetration. Considering that the fleshy fruits meat used had a lycopene content of 450 mg per 100 g dw, percentage recoveries at 3 minute blending were of about 77%, 74% and 78% with hexane, ethyl acetate or the mixture hexane/acetone/ethanol 50:25:25 as the solvent (Table 3).

Lycopene Assay

Lycopene concentration in the extracting solvents was determined by spectrophotometric measurement at room temperature in the wavelength range 350–600nm. A double-beam UV–VIS spectrophotometer (Japan) and quartz cells of 1-cm path length were used. The absorption spectra of the extract,

independently of the solvent used and displayed the three characteristic peaks of lycopene at around 445, 472 and 503 nm (Figure 2). To minimise interference from other carotenoids measurements were made at 503 nm, using a molar extinction coefficient of $1.585 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$.

To further test the purity, structural analysis of the extract was performed. UV Spectrum is shown in Figure 2, the maximum wavelengths are 447.2, 473.2 and 504.2 nm, which is the maximum wavelengths of pure lycopene reported in the literatures.

Table 1: Moisture Content (Fruits Flesh Only)

Name of fruits	Fresh fruits (%)	After blending (%)
Tomato	83%	88%
Mango	78%	80%
Water melon	91%	94%
Papaya	75%	79%
Black berry	82%	84%

Table 2: Lycopene From Coloured Flesh Malaysian Fruits Extraction by Different Solvent System

Name of fruits (coloured flesh parts)	Water extraction mg/100g dw	Hexane mg/ 100g dw	Ethyl acetate mg/100g dw	Hexane/Acetone/ethanol (50:25:25), mg/100g dw
Tomato	280.00 ± 2.10	380.89 ± 1.90	340.45 ± 1.45	460.80 ± 2.50
Mango	281.25 ± 4.50	395.90 ± 3.25	375.75 ± 2.54	440.25 ± 1.99
Water melon*	50.55 ± 1.25	60.20 ± 4.20	65.45 ± 5.62	75.45 ± 4.32
Papaya	289.89 ± 2.50	355.45 ± 1.20	320.80 ± 3.54	410.45 ± 1.25
Black berry	215.25 ± 1.90	265.45 ± 1.05	270.89 ± 2.25	310.15 ± 2.10

dw: dry weight; * = mg/kg fresh wt.

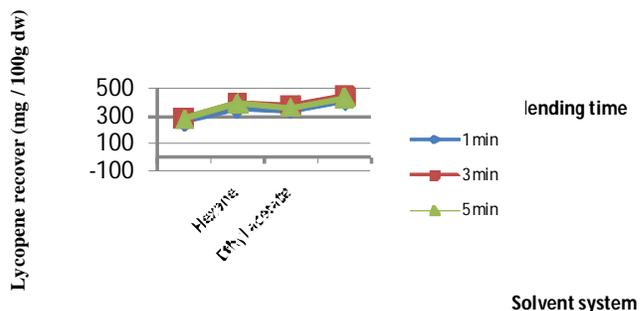
Table 3: Effect of Blending Time on Lycopene Extraction

Name of fruits (coloured flesh parts)	Water extraction mg/100g dw	Hexane mg/ 100g dw	Ethyl acetate mg/100g dw	Hexane/Acetone/ethanol (50:25:25), mg/100g dw
Tomato				
Blending time (1min)	210.25	350.34	310.12	430.35
Blending time (3min)	275.34	380.21	340.89	460.67
Blending time (5min)	265.78	375.25	335.34	455.12
Mango				
Blending time (1min)	260.33	360.23	345.45	410.12
Blending time (3min)	281.34	395.24	375.78	440.67
Blending time (5min)	275.12	390.25	365.38	435.23
Water melon*				
Blending time (1min)	35.45	40.15	50.24	65.45
Blending time (3min)	50.85	60.45	65.65	75.90
Blending time (5min)	48.23	55.53	55.42	65.12
Papaya				
Blending time (1min)	256.14	345.36	290.24	370.57
Blending time (3min)	289.69	355.57	320.70	410.78
Blending time (5min)	280.24	350.12	315.43	498.45
Black berry				
Blending time (1min)	195.43	245.42	220.13	280.68
Blending time (3min)	215.56	265.89	235.36	310.69
Blending time (5min)	205.38	260.27	230.28	300.13

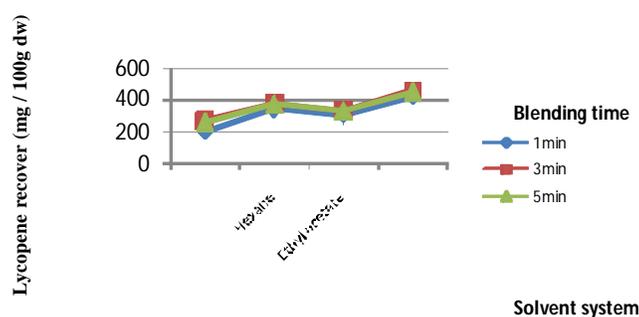
dw: Dry Weight; * = mg/kg fresh wt.

Effect of Blending Time on Lycopene Extraction

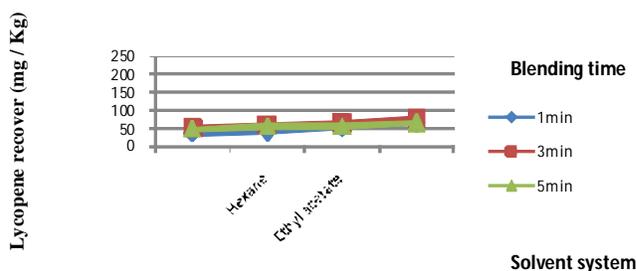
Tomato



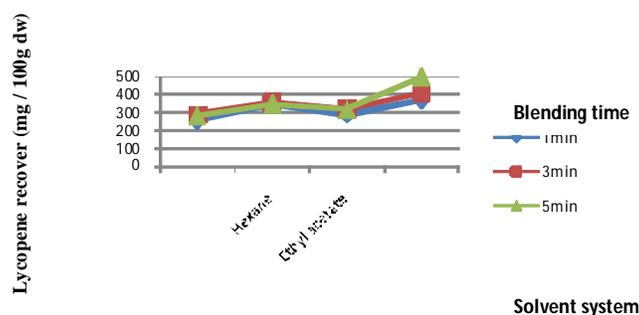
Mango



Water melon



Papaya



Black berry

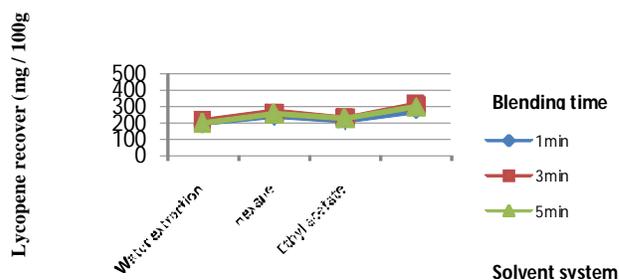


Table 4: Effect of Number of Extraction on Lycopene Recover (mg / 100g dw)

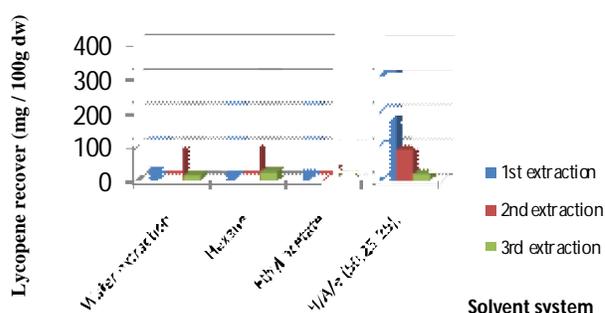
Name of fruits (coloured flesh parts)	Water extraction mg/100g dw	Hexane mg/ 100g dw	Ethyl acetate mg/100g dw	Hexane/Acetone/ethanol (50:25:25), mg/100g dw
Tomato				
First extraction	180.25 (64.3%)	260.39(69%)	240.12(73%)	350.35(76%)
Second extraction	84.34 (30.1%)	90.21(24%)	70.89(22%)	90.67(19.6%)
Third extraction	15.78 (5.6%)	25.25 (7%)	15.34(5%)	20.12(4.4%)
Mango				
First extraction	185.33(66%)	280.23(71%)	265.95(75%)	325.12(74%)
Second extraction	81.34(29%)	95.24(24%)	85.58(21%)	90.67(21%)
Third extraction	12.12(4.3%)	20.25(5%)	25.98(4%)	25.23(5.7%)
Water melon*				
First extraction	32.45(64.1%)	43.15(71%)	45.24(69%)	55.45(73%)
Second extraction	12.85(25.7%)	12.45(21%)	12.65(19.5%)	13.90(17.5%)

Third extraction	3.23(6.5%)	6.53(9%)	7.42 (11.4%)	7.12(9.4%)
Papaya				
First extraction	167.94(58.3%)	235.36(67%)	220.24(67%)	290.57(71%)
Second extraction	99.69(34.8%)	100.57(29%)	90.70(28%)	100.78(25%)
Third extraction	20.24(7%)	15.12(4%)	15.43(5%)	18.45(4.5%)
Black berry				
First extraction	130.43(60.6%)	175.42(67%)	180.13(70%)	235.68(75%)
Second extraction	70.56(32.7%)	65.89(25%)	65.36(25%)	65.69(21%)
Third extraction	15.38(7.1%)	20.27(8%)	10.28(4%)	10.13(3%)

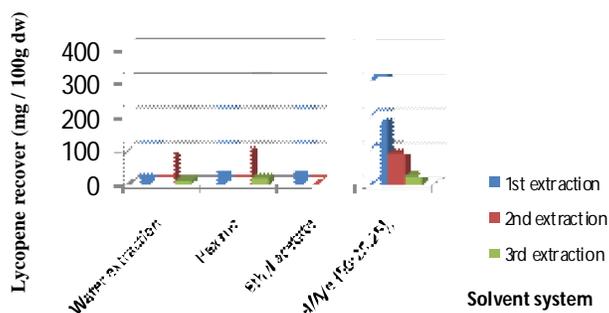
dw: dry weight. * mg/kg fresh wt.

Effect of Number of Extraction on Lycopene Recover (mg / 100g dw)

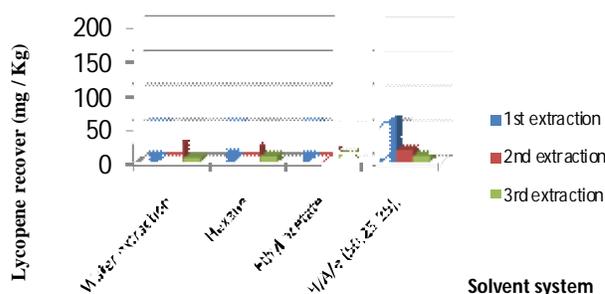
Tomato



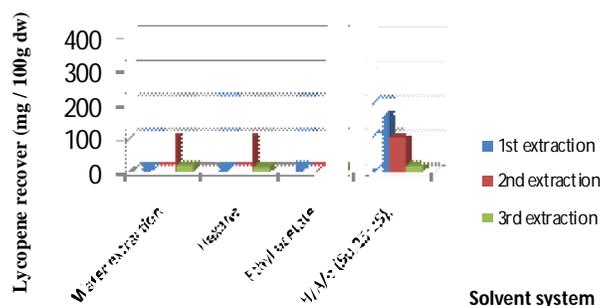
Mango



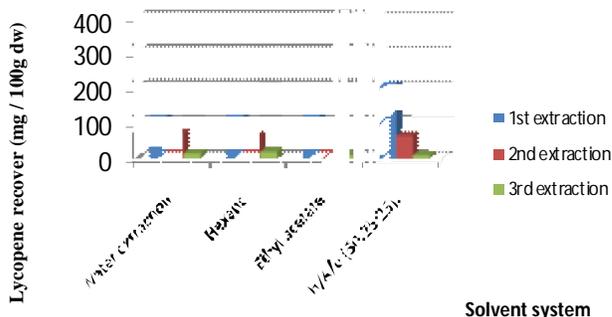
Water melon



Papaya



Black berry



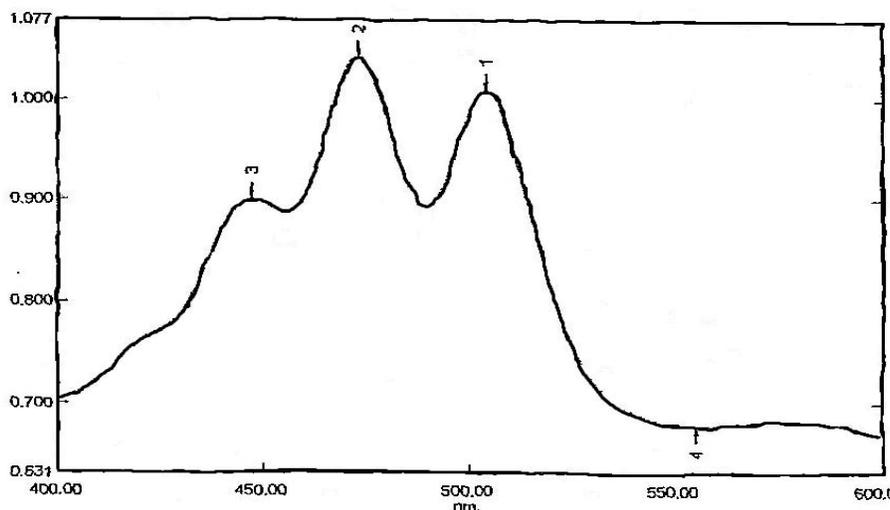


Figure 2: Visible Spectrum of Extracted Lycopene Crystals in Ethanol After Three Times Purification

CONCLUSIONS

To our knowledge, it was the first report on separation and quantisation of lycopene from Malaysian all season coloured fleshy fruits (Mango, tomato, water melon, black berry, and papaya). All fruits can be a good natural source of lycopene. This study describes a rapid and inexpensive way to estimate the lycopene of certain Malaysian fleshy fruits naturally rich in lycopene. We have demonstrated with fruits that the amount of lycopene in tissue can be determined reliably with a method that employs only 20% of the organic solvents used in conventional spectrophotometric assays. As the same time, the reduced volume extraction method gives statistically identical results with conventional extraction procedures such as those of [24]. In this work an extractive process is described that could be easily scaled-up to

become an industrial application for the production of very high grade of purity (98%) all-trans lycopene, whose demand is constantly increasing. The average recovery of lycopene from coloured fleshy fruits varies between 8-14% (w/w) in respect of chemical extraction at purity of 98% at the optimum extraction time. The material obtained after the extraction of the lycopene e.g. the exhausted fruits-waste residues can be easily dried and used in agriculture or as feeding for cattle or animals after the recovery of a high economical value molecule like lycopene. The pure all-trans lycopene extracted makes it possible to use it, at a known dosage, as a drug and not only as a food integrator. Of course, more simple, new, and environment friendly sorbent such as mono and bio materials can be used in the isolation and purification of lycopene from coloured fleshy fruits.

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