

Process Development for Maximum Lycopene Production from Selected Fruit Waste and its Antioxidant and Antiradical Activity

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Abstract

Lycopene, one of the most widely used carotenoid is an efficient antioxidant and singlet oxygen quencher. Increasing demand of lycopene in the nutraceutical and drug industry has directed the researchers to produce lycopene with cost effective methods in large scale to meet the growing demand. Thermal processing liberates this carotenoid from complexes with proteins and thus increases its bioaccessibility. The comparison of lycopene content was explored amongst four fruit peels; guava, papaya, watermelon and red dragon fruit to choose for the best source. Lycopene content was measured using both UV-vis spectrophotometer and identified using high performance liquid chromatography (HPLC) Papaya, a tropical fruit showed tremendous potential as an alternative source and was selected to conduct further investigation. Response surface methodology (RSM) using faced centered composite design (FCCCD) was applied to study the interaction between the most contributing factors i.e., temperature, time and solid-solvent ratio with maximum lycopene yield of 103.1 mg/kg, the DPPH and FRAP equals to 81.85% and 836.46 $\mu\text{M Fe(II)/L}$ respectively and a higher TPC of 1735.1 mg/L GAE at temperature 120°C, time of 5 hours in a solid-solvent ratio of 1:40 g/ml. While lycopene yield of 74.538 mg/kg exhibits DPPH scavenging activity of 91.14%; FRAP value of 954 $\mu\text{M Fe(II)/L}$ and TPC content equals 1409.42 mg/L GAE at a temperature of 120°C for 4 hours extraction time with a solid-solvent ratio of 1:30 g/ml. The lycopene oleoresin was saponified using a mixture of propylene glycol and aqueous alkali to obtain lycopene crystals. The substantially pure lycopene crystals so obtained are fit for human consumption and were identified with High Performance Liquid Chromatography revealing that the major constituents of the lycopene oleoresin after saponification were lycopene and β -carotene which constitute 69.879% and 30.121% of the total oleoresin respectively.

Keywords: Lycopene; Carotenoid; Oleoresin; Papaya; Antioxidant; FRAP; DPPH

Introduction

By products derived from food processing are considered highly advantageous for their development as nutraceuticals and food ingredients, additives, functional fruits, and are also used in the cosmetic industry due to the various bioactive components and colour pigments present in them. The natural pigments which impart red, orange and yellow colour in a wide variety of plants and fruits are known as carotenoids and comprise of various phytochemicals usually found in the food matrix such as β -carotene (orange), lycopene (red), lutein (yellowish-green), chlorophyll (green) and anthocyanin (blue-purple) [1-3].

Amongst these carotenoids, one of the most significant and abundant carotenoid found in nature is lycopene which has gained attention among nutritionists. Lycopene is mainly consumed through fruits and vegetables in our diet; with a primary source being tomato [4,5]. It is a natural pigment which is red in color and is found in fruits namely pink guava (115 mg/kg), papaya (500-600 mg/kg), watermelon (500-600 mg/kg), grapefruit (14.19 mg/kg), red dragon peel (73.00 mg/kg) [6-10].

Lycopene is known to have beneficial effects on human health due to its ability to act as a potent antioxidant and a scavenger of free radicals which is often associated with carcinogenesis. According to Leticia et al. [11] and Imrhan and Basu [12], *in vitro* studies have shown lycopene to be twice as potent as β -carotene and ten times that of α -tocopherol in terms of its singlet oxygen quenching ability. It may also interfere with oxidative damage to DNA and lipoproteins and inhibit the oxidation of LDL cholesterol. Epidemiological studies have revealed that the consumption of lycopene is inversely associated with the risk of atherosclerosis [13], cardiovascular disease [14-19], some cancer typologies [20-23], cognitive impairment [24], osteoporosis

[25] and other diseases. All the evidence is helpful for an understanding of the antioxidant role that lycopene can play.

Food processing by products from oranges, mango, guava, papaya, watermelon, red dragon fruit (pittaya) and also vegetables including tomato and carrots are potential sources of these functional foods. Recycling of these by-products can be highly beneficial based on both environmental point of view as well as for the health benefit derived from the recovery of bio active compounds. With the increasing demand of lycopene in the current market, numerous studies have focused on utilization of the waste products such as tomato pomace for lycopene extraction and in the development of nutraceuticals and functional products [4,26]. However, studies on other fruit sources for the extraction of lycopene were scarce and alternative cost effective sources for the production of natural lycopene are warranted. Therefore, our main aim in this study was to investigate the possibility of utilising unused parts of most potential fruits as lycopene source and establish its antioxidant activity. The current study discovered a new source of lycopene which can pose many therapeutic uses. Temperature has been established as one of the most important characteristic for increased lycopene production, as it improves the availability of lycopene

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from the cell wall and helps to remove moisture without destructing lycopene or other nutrients present [27-30]. With the acceptance of lycopene as a potent antioxidant and radical chelating agent, it can give a new dimension to the current medicinal usage of lycopene.

Methods

Sample preparation

The main experimental materials used for this study were fruit wastes, namely papaya, watermelon, guava and red dragon fruit peel which were obtained from a local fruit shop located in the vicinity. The fruit skin peels were collected from a local fruit shop located in the International Islamic University Malaysia (IIUM) and the separated peel were freeze dried until a constant weight was obtained. The dried samples were then ground using a grinder machine to get a uniform size of the sample. Samples were kept in airtight containers and stored at -20°C until further analysis. All the experimental procedures were carried out under dim light.

Extraction process

Lycopene extraction was carried out as per a method developed by Fish et al. [31]. The extraction, handling and analysis of lycopene must be carried out under controlled environmental conditions to minimize oxidative degradation and isomer formation. Exposure of lycopene to light should be avoided; and only gold, yellow or red light should be used. After heat treatment at desired temperatures and time period, sample (1 gm) was extracted using solvent (hexane, acetone and alcohol in the ratio of 2:1:1) containing 0.05% (w/v) butylated hydroxytoluene (BHT) [27]. Antioxidants such as butylated hydroxytoluene (BHT) were employed in solvents used for extraction such as to avoid oxidation and isomerisation reactions [32]. Cold distilled water (15 ml) was added to the mixture and the suspension was agitated at 200 rpm for 8 minutes. The solution was then allowed to stand at room temperature for 15 min for separation of polar and non-polar layers. The nonpolar supernatant hexane layer containing lycopene was collected in an amber flask and read in 1 cm path length quartz cuvette at 503 nm using UV-vis spectrophotometer with hexane as the blank.

Determination of the total lycopene content

The lycopene content was estimated as mg/kg fruit skin peels based on the following Equation (1).

$$\text{Lycopene (mg/kg)} = \frac{A503}{17.2 \times 10^4 \text{ M}^{-1} \text{ cm}} \times \frac{536.9 \text{ g}}{\text{mol}} \times \frac{10^3 \text{ mg}}{1 \text{ g}} \times \frac{10 \text{ ml}}{\text{kg sample}} \times \frac{1 \text{ L}}{10^3 \text{ mL}} = \frac{A503 \times 31.2}{\text{kg sample}}$$

Where $A503$ is the absorbance of the hexane layer containing lycopene

The molecular weight of lycopene is 536.9 g/mol, molar extinction coefficient for lycopene in hexane is $17.2 \times 10^4 \text{ M}^{-1} \text{ cm}$. Most of the other extinction coefficients reported for lycopene are subsequently within this range. Working with values of lycopene content expressed in terms of mg/kg (or, the equivalent, mg/g) makes data handling easier and is a unit of concentration commonly used in the literature.

Screening of the fruit peel for total lycopene content

Screening of the dried fruit peels including guava, watermelon, red dragon fruit and papaya was done in order to determine which source has the maximum lycopene content and depicts the maximum antioxidant activity under controlled conditions. Lycopene from the freeze dried ground samples of all the fruit peels were extracted using a mixture of solvents namely; Hexane, Acetone and Ethanol in the ratio

of 2:1:1 in Erlenmeyer flasks. The first batch of extraction from samples was conducted without heat treatment and the volume of solvent used was 20 ml with 1 gram of ground fruit peels. The second batch of fruit peels was subjected to a temperature of 90°C for 3 hours to investigate the effect of heat on lycopene availability [4,31-33]. All the extraction samples were conducted in three replicates and the final extract yield was separated from the hexane layer and the amount of lycopene was determined for each sample. The best source was chosen based on the highest lycopene content. The chosen fruit peel source was used throughout the study for process parameter optimization.

Heat treatment of fruit peels

1 gram of each sample was transferred into flask, with desired sample volume, sealed such as to avoid any solvent or sample loss and wrapped with aluminium foil to protect them from light degradation. Temperature and heating time were assigned according to the experimental design. The wrapped flasks were placed into an oil bath and were subjected to heat at 90°C , 105°C and 120°C for a time range of 3-5 hours each [33]. Temperature was measured directly in the samples using a thermocouple. Experiments were replicated three times. The heat treated samples were then subjected to solvent extraction of lycopene as stated by the method described by Fish et al. [31].

Purification of the lycopene containing oleoresin

The lycopene containing oleoresin was saponified using propylene glycol and an aqueous alkali to form lycopene crystals according to a method developed by Ausich et al. [34] referred in a patent (No. WO 5,858,700) with slight modifications. Based on HPLC results, the resulting crystals obtained after purification contain approximately 65%-70% of the total carotenoids present in the lycopene containing oleoresin, and are considered substantially pure lycopene. The considerably pure lycopene obtained after saponification are rendered free of any residual toxic organic solvents besides propylene glycol or other toxic compounds, and is deemed fit for human consumption.

Evaluation of the antioxidant activity

Ferric Reducing Antioxidant Power (FRAP ASSAY): The ferric reducing antioxidant power (FRAP) assay is used to measure the ability of antioxidant capacity to reduce the Fe^{+++} /tripyridyl-s-triazine (TPTZ) complex, to the ferrous form. The procedure was based on the work recently published by a research group. After freshly preparing the FRAP reagent, 100 μL of the extract was added to 3.0 ml of FRAP reagent and the reaction mixture was incubated in a water bath for 30 minutes at 37°C . An increase in absorbance was measured using UV-VIS spectrophotometer at 593 nm.

DPPH radical scavenging (Antioxidant Assay): 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was determined by the suggested method [35] with slight modification. 100 μL of the test solution was mixed with 3.9 ml of ethanolic DPPH (60 μM) in a 15 ml test tube that had been wrapped with aluminium foil to avoid photo degradation. After vortexing the solution for 30 seconds, the mixture was then allowed to stand for 30 minutes at room temperature in dark environment. An absolute ethanol was solely used to prepare the blank. The absorbance of blank, control and the sample were measured at 517 nm by using UV-VIS spectrophotometer. The scavenging activity was expressed as percentage of inhibition, which was calculated according to the following Equation:

$$\text{Scavenging activity (\%)} = 1 - \frac{\text{ABS } 517 \text{ sample}}{\text{ABS } 517 \text{ control}} \times 100$$

Where, *ABS517* sample refers to the absorbance of samples containing the extracts after 60 min and *ABS517 control* refers to the absorbance of a sample containing ethanol instead of extract.

Results and Discussion

Screening of the fruits for highest lycopene content

The lycopene obtained from simple solvent based extraction depicted papaya to be the best source as compared to watermelon, red dragon fruit or guava. Eight independent experiments were conducted on all the four fruit varieties with a volume ratio of 1:20 for 3 hours at two varying temperature conditions; 27°C and 90°C. The lycopene yield for the fruits papaya, watermelon, red dragon and guava peel are 25.30, 11.14, 8.4 and 10.7 at 27°C respectively. However, the results obtained from the extraction process using heat treatment at 90°C for papaya, watermelon, red dragon and guava peel are 36.816, 13.3, 6 and 11.87 mg/kg respectively. The results obtained for watermelon, red dragon fruit and guava peels were low compared to previous findings as fruit peels were used for extraction while previous findings were based on fruit itself. However, for papaya peels, the content was considerably higher which can be attributed to the heat treatment of the peels. Previous studies [27-30] have suggested that heat treatment alone can potentially improve the lycopene availability by rupturing the cell walls, thus weakening the level of interaction between lycopene and the tissue matrix of the samples which was confirmed in our results.

Setiawan et al. [6] found that the amount of lycopene in guava, papaya and red watermelon ranged between 115, 575 and 1175 mg/kg fresh weight (FW) respectively. In other study conducted by Charoensiri et al. [7], similar results were reported. Yano et al. [9] stated that after comparing the amount of lycopene present in lycopene-rich tropical fruits, pink guava depicted a higher lycopene content than papaya (200 mg/kg) and lower than that of watermelon (53 mg/kg). Moreover, a study conducted by Puentes et al. [36] stated that the most widely available carotenoid in red fleshed watermelons was lycopene (84-97% of total carotenoids), as it is present predominantly in the flesh of the fruit. While lycopene is abundant in pink guava fleshed fruit, ripe papayas and red fleshed watermelons due to increase in the biosynthesis of these compounds during fruit ripening [8,9], a lower content of lycopene here could be attributed due to a different variety of guava fruit was used (white fleshed) and also due to insignificant lycopene content in the skin of the fruits [37]. The difference in the previous findings and here could also depend on the method of extraction, storage of samples and fruit ripening.

Optimization of lycopene extraction from dried papaya peels

The dehydrated papaya peels were used for the extraction of lycopene. The extraction process was standardized for the maximum recovery of the pigment, using response surface methodology using three independent variables and their levels were selected based on Face Central Composite Design. It was observed that the lycopene content varies between 59.712 to 103.1 mg/kg from papaya peels (Table 1). A similar profile for lycopene extraction was reported in previous studies done on papaya [38-40]. Previous studies on lycopene has been done on ripe papaya fruit and the highest values were found by Sancho et al. [41] (327 mg/kg FW); Setiawan et al. [6] showed that highest level of lycopene from ripe papaya was estimated to be as high as 575 mg/kg fresh weight (FW) which were in correlation with the findings of Charoensiri et al. [7], with a lycopene content of 669 mg/kg fresh weight (FW) in ripe papayas. These differences between our finding and the previous researches could be attributed to the papaya peels

Run	Temp °C	Time hours	Solid-Solvent g/mL	Response		
				Lycopene mg/kg	FRAP $\mu\text{M Fe(II)/L}$	DPPH %
1	90	3	1.20	71.816	180	67.9
2	120	3	1.20	61.51	270.13	90.7
3	90	5	1.20	59.712	217.8	73.8
4	120	5	1.20	72.34	1068.45	85.57
5	90	3	1.40	81.3072	332.13	67.717
6	120	3	1.40	75.91	698.63	88.41
7	90	5	1.40	68.297	232.6	53.74
8	120	5	1.40	103.1	836.46	81.85
9	90	4	1.30	77.154	732.12	63.24
10	120	4	1.30	74.538	954	91.14
11	105	3	1.30	82.48	1070.06	86.65
12	105	5	1.30	84.91	782.8	84.58
13	105	4	1.20	75.73	615.87	75.9
14	105	4	1.40	83.153	827.18	90.204
15	105	4	1.30	88.38	756.6	84.75
16	105	4	1.30	89.18	802.66	84.96
17	105	4	1.30	89.24	764.53	83.73
18	105	4	1.30	89.92	741	84.98
19	105	4	1.30	88.86	737.23	84.175

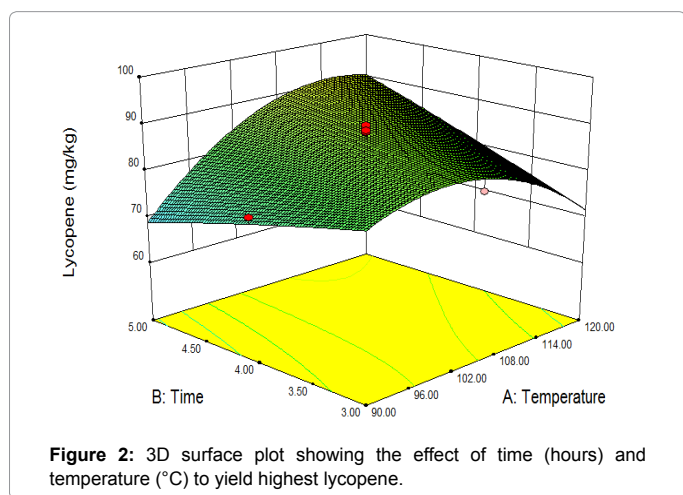
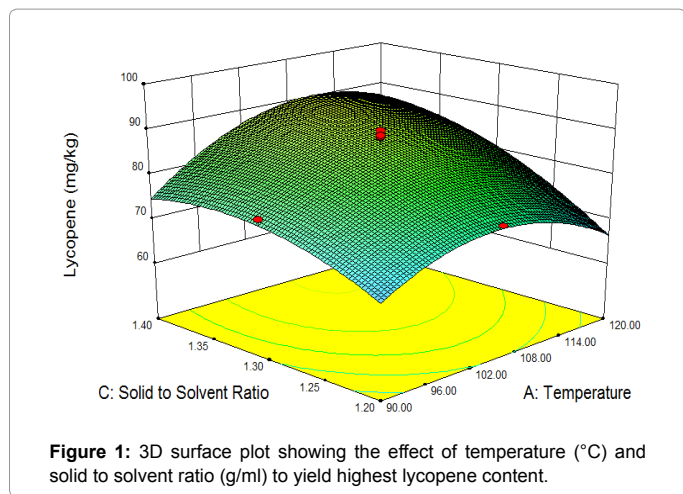
Table 1: Correlation between Lycopene, DPPH and FRAP responses.

which were used as the source instead of the fruit flesh itself. It could also be due to agricultural practices, exposure to sunlight, cultivation area, ripeness, postharvest handling, and methodology employed for analysis [42-44].

Figure 1 shows that highest lycopene content obtained at a temperature of 110°C and the ratio of solid to solvent used is 1:40 g/ml while time was kept constant at 5 hours. A similar profile for lycopene extraction was reported in previous studies done on papaya [38-40]. Previous studies on lycopene has been done on ripe papaya fruit and the highest values were found by Sancho et al. [41] in fruit of variety RS4 (327 mg/kg FW).

The surface plot showed in Figure 2 indicate that an increase in the temperature from 90°C to 108°C but reduction in time to 4 hours can also result in a higher total lycopene yield. This shows that lycopene compounds present in the peel are heat sensitive, thus handling them at a temperature lower than 110°C would result in a higher yield. In fact, many authors agree that with an increased temperature favors the extraction process thereby enhancing the solubility of solute and diffusion coefficient. However, it has also been reported that excess heat can lead to degradation of the compound [27-30,33,45].

The duration of thermal processing plays an important role in lycopene accessibility analysis. Lin and Chen [45] showed that with an increased temperature favors the lycopene bioavailability. Shi and Le Maguer [20] and Vanden et al. [46] stated that heat processing can breakdown cellular walls, rupture chromoplast membranes and decrease cellular integrity leading to an increased bio accessibility of various carotenoids especially lycopene. Chengwai et al. [35] explained the effect of heat processing on lycopene availability and concluded that at temperatures below 100°C, the stability was not affected whereas heating at 120°C and 140°C increased isomerization of lycopene and may have caused degradation of total lycopene and cis-isomers present. Similarly, Shi et al. [28] reported that isomerisation happens at a temperature of 60°C for 5 hours; however, they obtained higher lycopene content by heating a tomato puree matrix at 100°C or 120°C for 2 hours. According to Topal et al. [47] and Gomez-Prieto et al. [48]



the extraction yield of lycopene from tomato by products increased with temperature and time to extract lycopene from skins and from skin + pulp. However, Vagi et al. [48] indicated that only temperature had a significant effect ($p < 0.05$) on the yield. According to this data, it shows that lycopene availability increases only during the first 4 hours of heating and decreased during heating times above 4 hours. However, study done by Ekorong et al. [49] showed a reduced level in the polyphenol compounds obtained from oven-dried mango seed kernels which could have resulted from the degradation of phenolic compounds at high temperatures, due to chemical, enzymatic, or thermal decomposition.

Kaur et al. [4] showed that the high lycopene yield was achieved with a solvent meal ratio of 1:30 g/ml for production of lycopene from tomato pomace. Fish et al. [31] used a low volume hexane extraction method with a solvent ratio of 1: 20 g/ml and the lycopene content ranged between 6.6 to 490 mg/kg on a fresh weight basis. However, Lin and Chen [43] reported that solid-solvent ratio showed no major influence on lycopene availability and isomerisation. Choudhari and Ananthanarayan [32] used tomato skin as the source of lycopene and the solid-solvent ratio was maintained in between 1:20 to 1:40 g/ml and highest value was obtained at 192 mg/kg fresh weight basis at solvent volume of 1:30 g/ml. The effect of solid-solvent ratio as extraction variable was confirmed by Nunes and Mercadante [50] (Figures 1 and 2).

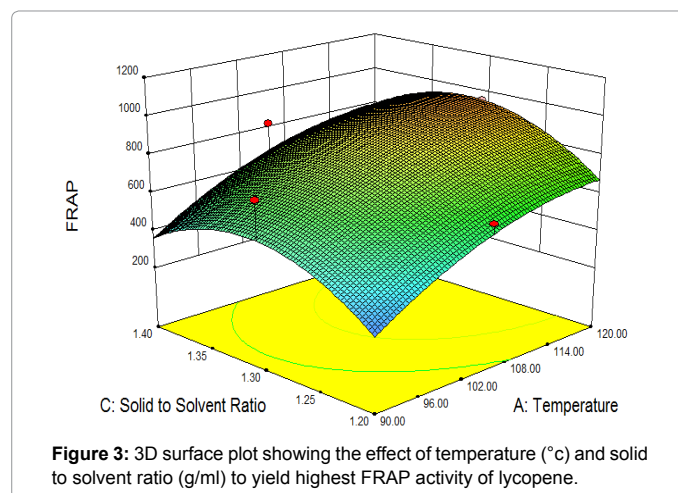
Antioxidant activity

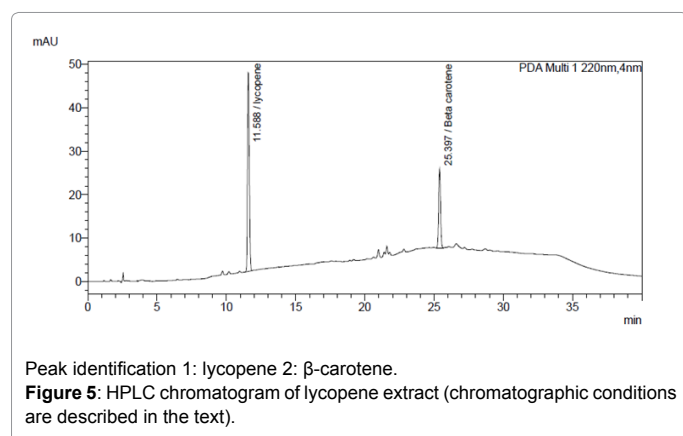
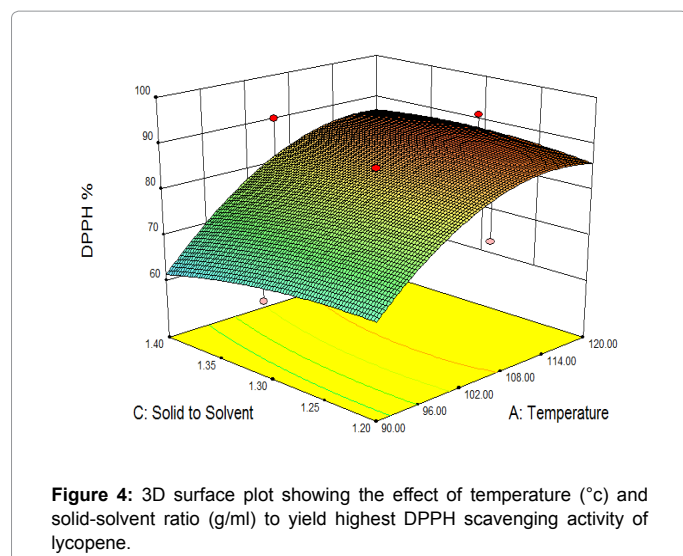
Ferric Reducing Assay (FRAP): The highest antioxidant reducing power (1070.06 $\mu\text{M Fe(II)/L}$) for the lycopene sample was obtained at a temperature of 105°C and solid-solvent ratio of 1:30 g/ml while maintaining the time of extraction as 3 hours as shown in Figure 3. Although, it has been reported that antioxidants detected by FRAP are limited to water-soluble ones and that carotenoids, having no ferric reducing ability, should not react with this method [51], however, excellent results were obtained considering the FRAP assay at 836.46 $\mu\text{M Fe(II)/l}$ with total lycopene 103.1 mg/kg content. According to Mortensen and Skibsted [52] in the group of carotenes, only lycopene is an effective ferric reducing compound, hence confirming our results. The ferric reducing activity is mainly influenced by the size of the conjugated double bond (CDB) system. In lycopene, an acyclic carotenoid with 11 CDB, the orbital overlap in the chromophore is sufficient to form a stable carotenoid radical and consequently display a high FRAP activity.

Results here indicated that mobilization of active compounds in the extract may occur up to a certain level followed by their possible degradation due to an increased temperature. It may also be stated that an increased temperature may favor extraction of lycopene by enhancing solubility of antioxidant in the solvent. Additionally, antioxidant activity varies amongst foods containing lycopene. In one study, researchers found higher antioxidant activity in heat processed sources compared with fresh stock, presumably because processing improved the extraction and activity of antioxidant compounds, thereby increasing the FRAP value [53].

DPPH radical scavenging assay: While the FRAP assay measures the ability of antioxidants to reduce a ferric tripyridyltriazine complex. The main limitation of this method is that the measured reducing capacity does not necessarily reflect antioxidant activity [54]. DPPH assays are very popular as they are operationally simple. DPPH is a free radical compound that has been widely used to determine the free radical scavenging ability of various samples including lycopene.

The highest scavenging activity (91.14 %) was obtained at a temperature of 120°C for 4 hours and the amount of solvent used was 30 ml as shown in Figure 4. Zhang et al. [20] did an antioxidant study on papaya fruit at various concentrations and found that the scavenging activity of the fraction P1 was lower ($8.59 \pm 0.93\%$) at 0.023 mg/ml and, at a concentration of 3.57 mg/ml, reached a plateau of $78.5 \pm 0.36\%$. These values are higher as reported by Ito et al. [16] of around 22% and





by Liu et al. [55] of less than 9% for free radical scavenging activity. Still, according to Liu et al. [55] lycopene presents a curious direct relationship between its concentration and its capacity to sequester free radicals (DPPH). Also, according to Shi and Le Maguer [27] the antioxidant activity of lycopene is affected by its isomerisation, thereby depending on the thermal processing conditions. Sesso et al. [19] also reported that in the organic fraction from fruits, the main component responsible for sequestering free radicals is lycopene (Figures 3 and 4).

Purification of lycopene by saponification: Structure of carotenoids such as lycopene is solely responsible for imparting their colour and biological activities. Therefore, conclusive identification of carotenoids is an integral part of their analysis. The extract can have a varied composition of carotenoids both qualitatively and quantitatively and hence purification and identification of desired carotenoid i.e., lycopene can therefore be a delicate task [56]. The purification of the oleoresin is carried out by saponification using an alkali before performing the qualitative analysis by High Performance Liquid Chromatography (HPLC).

Marelli de Souza et al. [57] reported that the carotenoid composition of papaya fruit constituted of lycopene and β -carotene and lycopene was observed to be the abundant fraction representing 65% of the total carotenoids. Similarly, Rivera-Pastrana et al. [38] observed a similar profile of carotenoids in another variety of papaya namely "Mardol".

Therefore, the lycopene extract obtained from the papaya peels might contain other carotenoids including β -carotene which is soluble in the hexane layer of the extract and must be separated in order to get pure lycopene.

Qualitative identification of lycopene by high performance liquid chromatography: The most suitable mobile phase system was established after several tests and consist in: component "A" Acetonitrile-water (1:1, v/v) and component B Methanol. A C18 column and a binary gradient were used for carotenoids analysis. The gradient changed from 10% to 80% solvent B, as follows: 0-3 min 100% solvent B, 3-4 min 20% solvent B, 4-5 min 30% solvent B, 5-8 min 35% solvent B, 8-12 min 45% solvent B, 12-15 min 70% solvent B, 15-20 min 80% solvent B, 20-28 min 70% solvent B, 28-40 min 60% solvent B and the column re-equilibrated with 0% solvent B for 15 minute [58,59].

The mobile phase flow rate was 0.6 ml/min for the analysis. The column temperature was maintained at 32°C and the carotenoids elution was monitored at 220 nm for β -carotene and lycopene. UV spectra were recorded with a DAD detector from 200 to 700 nm and they were lycopene and β -carotene as shown in Figure 3.

Identification of carotenoids was carried out by comparison of the HPLC retention times with corresponding standards and co-chromatography with added standards. The chromatograph showed two isolated compounds i.e., lycopene and β -carotene at retention times of 11.588 and 25.397 minutes. Confirmation with Mass Spectrometry (MS) and Nuclear Magnetic Resonance (NMR) of the purified compounds can give an extensive detail of the compounds present (Figure 5).

Conclusion

Lycopene was extracted from papaya fruit peels after screening four different fruit peels for the highest lycopene content. Nineteen sets of experiments were conducted on selected combinations of solid to solvent ratio, temperature and time. The experimental value of lycopene yield (response variable) varied from 59.712 to 103.1 mg/kg. The second order model developed for lycopene content exhibited non-significant lack of fit and a high value for the coefficient of determination (0.9103). The surface graphs indicated that maximum lycopene yield was obtained by extracting papaya peels at a solid to solvent ratio of 1:40, temperature of 120°C and a time of 5 hours. The DPPH radical scavenging ability assay and the FRAP assay was carried out to examine the antioxidant activity of the lycopene extract which is crucial to protect the human body against free radicals that may have pathological effect. The highest percentage for DPPH was set at 91.14% and the highest FRAP value obtained was 954 μ M Fe(II)/L. The lycopene oleoresin was subjected to saponification to achieve a pure lycopene sample using propylene glycol and an alkali (KOH) which was assessed for all impurities and identification of the desired compound i.e., lycopene by HPLC. The results confirmed that the extract contained high level of lycopene and small traces of β -carotene which was removed after the purification step.

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