Enhanced Knowledge in Sciences and Technology Vol. 1 No. 2 (2021) 132-142

EKST

Homepage: http://publisher.uthm.edu.my/periodicals/index.php/ekst
e-ISSN : 2773-6385

Evaluation of Stable Pigment Extracted from Red Tomato (*Solanum lycopersicon*)

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DOI: https://doi.org/10.30880/ekst.2021.01.02.016
Received 24 May 2021; Accepted 03 July 2021; Available online 29 July 2021

Abstract: Lycopene is a bright red carotenoid pigment and a phytochemical compound that can be discovered in the tomatoes. The aim of this study is to produce the stable colouring towards temperature and pH by using suitable method of extraction and its potential to be alternative natural food colourant. The lycopene pigment was detected at wavelength 470 and 471 nm by using UV-Vis spectroscopy analysis. In this study, the lycopene was extracted from red tomato (*Solanum lycopersicon*) by using hexane and acetone as the solvent. The maceration method has been applied in order to maintain the stability of the pigment where 173.88 mg/kg tomato pomace obtained from this study. Lycopene pigment was instable at alkaline solution due to their chemical structure and at temperature 50 and 60 °C where the great loss of this pigment is noticed at both parameters. Meanwhile, the antioxidant activities (66.35 %) and antimicrobial properties towards gram-positive bacteria were confirmed to be present inside the lycopene pigment.

Keywords: Lycopene, Extraction, Stability, Antioxidant, Antimicrobial.

1. Introduction

Pigments are chemical compound present inside the organism that provides attractive colours to them. It plays a vital role in physiological criteria, involves in light capturing photosystem and acts as a medium to attract pollinator for dispersing mechanism. Pigments can be classified base on their origin either as natural (obtained from plants, animals, fungi and microorganism), synthetic (obtained from laboratories and chemical production) or inorganic pigment (can be found in nature or reproduced by synthesis) [1].

The synthetic pigment is a by-product of chemical synthesis, originally from coal-tar. This type of pigment is more popular among the industry compare to natural pigment as they are low in cost, high colour intensity, high stability, long-lasting manners and produce more uniformity to the food products. They can be subdivided into two major classes which are azo food and non-azo food pigment. Colours

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attributes are fundamental for quality assessment that influence consumer preferences [1]. In the food processing industry, manufacturer tends to use synthetic pigment as their main sources of colouring due to their numerous advantages and more attractive than natural ones. Therefore, excessive usages of this colouring will associate with possible harmful effect on human health especially children [2].

Meanwhile, natural pigment is the pigment that can be obtained from plant sources (flowers, fruits and leaves) and animal origin (animal blood, muscle tissue and microbial sources) which is safer for consumption. Lycopene is a type of natural pigment classified under carotenoid with 11 conjugated double bonds that is abundant in tomato and its derivatives [3]. Saini *et al.* stated that the lycopene pigment that experience several processing steps will undergo several degradations via isomerization and oxidation especially, during thermal processing [4]. Therefore, the application of natural pigment is limited than the synthetic pigment because of its molecular instability if exposed to the environmental parameter, high cost and its required high concentration of pigment to increase the colour intensity [5]. Nevertheless, with these issued it contains bioactive compound likes antioxidant that will benefit to boost the health status of consumers.

This study focuses on the extraction of lycopene pigment from skin and mesocarp of red tomatoes (*Solanum lycopersicon*). The main objective is to produce stable natural colouring products from tomato fruit. Several parameters that affect the stability and physical properties of the pigment which is temperature, pH, antioxidant and antimicrobial properties were also studied.

2. Materials and Methods

2.1 Chemical and apparatus

The fresh tomato (*Solanum lycopersicon*) was purchased around the Muar, Johor local market. The solvent hexane and acetone used in this study were of analytical grade purities from Qrec and R&M Chemical respectively. The 2,2-Diphenyl-1-picrylhydrazyl was purchased from Sigma Aldrich Chemical Co. (St.Louis, German) and standard drug which is ampicillin sodium salt that acts as antibiotic in the antimicrobial assay was purchased from HIMedia Laboratories Pvt. Ltd. (Mumbai, India). The Mueller-Hinton agar was obtained from Laboratories Conda S.A. (Madrid, Spain) with bacterial culture, *Bacillus cereus* and *Serratia marcescens* SA30. UV-Vis spectral analysis has been conducted by using UV-VIS Spectrophotometer (U-3900H) with wavelength ranged is between 200 to 600 nm.

2.2 Sample preparation

The fresh ripe tomatoes sample were selected according to maturation. The red tomatoes were washed thoroughly by using tab water for removal of debris and sliced with a standardized thickness. Then, the tomato slices were placed in oven dryer (Memmert, German) at 80 °C for 2 hours and then shifted to 60 °C for another 6 hours [6]. The dried sample was collected and ground using food blender. Tomato powder was stored in a sealed plastic bag prior to further analysis [7].

2.3 Extraction of pigment

The lycopene pigment was extracted according to the method described by Rizk *et al.* [8]. The extraction was preceded by using the maceration method. Thus, this extraction process was allowed to be conducted at room temperature. The ground tomato sample were weighed in a beaker and 100 ml of solvent, hexane and acetone (60:40, v/v) was added. The mixture was stirred for 3 hours and allowed to stabilize. Next, the solution was homogenized for 1 minute and the mixture was then filtered through Whatman filter paper (Whatman No 1). After that, a separatory funnel was used for separation of non-polar hexane layer containing lipid residue with water-soluble fraction. The solvent was removed by reducing the pressure and the oily of lycopene extract was kept in glass bottle until the next analysis.

2.4 Determination of total lycopene

Lycopene content was determined by spectrophotometric method [9]. The absorbance spectrum was measured at 470 nm by UV-Vis spectrophotometer (Model U-3900 H, Hitachi, Japan). The lycopene content was calculated according to Eq. 1 as reported by Obadina *et al*. [10]:

$$\text{Lycopene(mg/kg)} = \frac{171.7 \text{ X } A_{470}}{W} \qquad \text{Eq. 1}$$

Where A_{470} = absorbance at 470nm, W = weight of the sample (kg)

2.5 Identification of lycopene: Ultraviolet-Visible (UV-Vis) Spectroscopy.

The carotenoid extracted from tomato were identified according to the method reported by Khamis *et al.* [11]. The lycopene was analysed by scanning in UV-Vis spectroscopy (Model U-3900 H, Hitachi, Japan). They were measured in a 1 cm path length quartz cuvette at 470 nm with hexane as a blank. A cuvette filled with hexane was allowed to run as a blank and filled the cuvette with three-quarter full of lycopene extract as sample.

2.6 Colour determination

The colour of an extracted sample was measured by hunter colour difference meter, MSEZ HunterLab (Virginia, USA). Before the colour measurement, the calibration of colorimeter was preceded by using white tiles. The L* represent for lightness index where; 0=black and 100=white, a* represent greenness and redness value where; >0= red and <0=green and b* denotes the intensity in yellow to blue where; >0=yellow and <0=blue. The data were analysed triplicate [12]. The colour parameter that was calculated are a*/b*, C* and h by using the Eq. 2,3 and 4 below:

$$\text{Redness to yellowness ratio (a*/b*)} = a^*/b^* \qquad \text{Eq. 2}$$

$$\text{Chroma (C*)} = \sqrt{(a^2 + b)^2} \qquad \text{Eq. 3}$$

$$\text{Hue (h)} = [\tan - 1\ (b^*/a^*)] \qquad \text{Eq. 4}$$

2.7 Stability of lycopene extracted from tomato

a) Effect of pH on the efficiency of lycopene pigment

A preliminary study is conducted to test the stability of lycopene pigment at different pH values ranging from pH 2 to 12 respectively for 30 minutes. The emulsion sample was prepared. The sample was dissolved in 20 ml of 2.0 M acetate buffer. The pH buffer was adjusted to pH 5. The modification of pH was done by adjusting to the desired final pH value by using either NaOH or HCl. The emulsion was transferred into the beaker and stored at ambient temperature. The absorbance of the sample was measured at λ=470 nm by using UV-Visible spectrophotometer. The percentage of colour loss was calculated. The experiment was conducted in triplicate to obtain the accurate result and data were presented in mean value with standard deviation [13].

b) Effect of temperature on the efficiency of lycopene pigment

A preliminary study was conducted to the effect of heat for lycopene extracted from red tomato at different temperature ranging from 40 to 60 °C for 30 minutes and the percentage of colour loss was calculated. The absorbance of the sample was measured at 470 nm with UV-Visible spectrophotometer. The experiment was conducted in triplicate to obtain the accurate result and data were presented in mean value with standard deviation [8].

2.8 Antioxidant activity testing of lycopene pigment

The antioxidant activity was evaluated according to the method proposed by Pu and Tang [14] with a modification. DPPH free radical scavenging activity of the pigment was measured by using DPPH radical scavenging method and percentage of scavenging activity was calculated. The sample was measured in a measuring cylinder and diluted with distilled water. The diluted sample was then mixed together with 3 ml of DPPH solution comprises from 2.4 mg of DPPH with 100 ml ethanol. The reaction mixture was allowed to react at room temperature in dark condition for 30 minutes. The absorbance was measured by using UV-Visible spectrophotometer (Model U-3900 H, Hitachi, Japan) at 517 nm. Ethanol serves as blank and the mixture of 2.5 ml ethanol plus 1 ml of DPPH radical solution will act as the control sample [15]. The experiment was repeated triplicate. The scavenging activity percentage (AA%) was calculated by using Eq 5:

$$AA\% = 100 - \left(\frac{(Abs\ sample - Abs\ blank)x100}{Abs\ control}\right) \qquad \text{Eq. 5}$$

2.9 Antimicrobial activity of lycopene pigment

The antimicrobial property of lycopene was evaluated by using an agar disk diffusion method [16]. The Muller-Hinton agar (Cronda, Madrid, Spain) and ampicillin (HIMEDIA, Mumbai, India) that act as positive control were used for this antimicrobial assay.

For this test, two types of bacteria, gram-positive bacterial strain (*Bacillus cereus*) and gram-negative bacteria strain (*Serratia marcescens* SA30) were used. The inoculum was prepared. This step was repeated by using *Serratia marcescens* SA30. All the isolates were spread on the Muller-Hinton agar and allowed to dry at room temperatures not more than 15 minutes. The stock solution for ampicillin was prepared for positive control in this assay. Immersed the sterilized disk (autoclave at 120 °C for 2 hours) into test compound; positive control (ampicillin), negative control (sterile distilled water) and lycopene sample. The disk that impregnated with test compound was placed on the solidified agar and pre-incubated the plate at room temperature for 1 hour. Then, all Muller-Hinton plate were incubated at 32 °C for 24 hours and the inhibition zone was measured

2.10 Statistical data

All the result obtain were reported in mean $\pm$ standard deviation.

3. Results and Discussion

3.1 Determination of total lycopene

The result indicates that the total lycopene contained in *Solanum lycopersicon* were varied from 285.19, 132.90 to 103.54 mg/kg of sample with average concentration was 173.88 mg/kg. The standard deviation of lycopene content in the sample was 97.51 mg/kg. The lycopene content in this experiment was slightly low compare to previous study where the average amount of lycopene obtained was ranged from 211.53 to 246.02 mg/kg [10]. The result by Zuoora [17] showed that the amount of lycopene extracted is 272$\pm$28 mg/100g of lycopene.

3.2 Identification of lycopene by Ultraviolet-Visible (UV-Vis) Spectroscopy.

UV-Vis spectrum of lycopene appears at three peaks which are located at 444, 470 and 471 nm. At wavelength 444, 470 and 471 nm the mean value is 0.2413, 2.8217 and 1.3133 respectively. Meanwhile, the standard deviation obtained in this experiment are 0.3441, 1.0127 and 1.3133 at respected wavelength. The main peak for lycopene in this experiment is at 470 nm. According to Anton and Barret [18], the lycopene has three peak of absorbance which is at 444, 471 and 503 nm but these three peaks are at a position of beta-carotene spectrum and other minor carotenoids that might cause a little interference to the data. In conclusion, the lycopene pigment was present inside the sample as two peaks

of the absorption spectrum were detected in the sample at the wavelength 470 with 471 nm. Meanwhile, a component at wavelength 444 nm was recognized as lutein that also present inside the sample but only at a lower concentration.

3.3 Colour determination

The technique applied in order to determine the colour parameter of sample were by using a colorimeter (MiniScan EZ, HunterLab, Virginia) for assessing the intensity of remaining pigment after the extraction process takes place. Table 3.1 shows the colour parameter of tomato powder and lycopene extract.

The average L* value for tomato powder is 43.317 which are higher than lycopene extract (5.540) thus it verified that tomato powder has a lighter colour intensity as the L* value is higher than the extracted product. Mean value for chroma a* in tomato powder and extract are 10.633 and 12.807 respectively. Based on the observation, the value a* for lycopene extract is higher from the powder forms. The higher a* value will indicate high redness intensity and the amount of lycopene pigment. Next, the mean value for chroma b* in tomato powder is slightly higher than in the extract which is 15.147 and 9.427 respectively. The b* value in colour scale will denote for the yellow colour as beta-carotene presence. The occurrence of beta-carotene inside the tomato powder are higher than in extract as it will be reduced through extraction process. Therefore, as the b* value is higher, the beta-carotene that presence inside the tomato powder is also higher [19].

The mean of a*/b* value was significantly lower for tomato powder which is 0.071 compared to 1.450 after the extraction process. The difference in redness between these two samples may be due to the effect of the extraction solvent. As the lycopene is a non-polar component, the extraction process will be enhanced by the aid of polar solvent which is acetone and n-hexane. According to Brandt *et al.* [20], the value of a*/b* ratio ranged from 1.0 to 1.18 will indicates the deep red colour intensity. It simultaneously shows the relationship between the colour intensity with the lycopene content. Thus, the higher the a*/b* ratio show the higher amount of lycopene pigment inside the sample. The result shows that the mean value for C* in powder (128.208) is lower than in lycopene extract (173.446). The mean for hue angle in powder is lower (0.0074) than in extract (0.0128). This shows that the tomato powder contains a brighter red colour than in the lycopene extract. Moreover, the hue angle with b* value is correlated as both parameters are associate with the amount of beta-carotene [19].

Table 3.1: Colour intensity of lycopene pigment

Sample	L*	a*	b*	a*/b*	Chroma (C*)	Hue (h)
Tomato powder	43.3167± 0.3296	10.6333± 0.7994	15.1467± 0.7315	0.7020± 1.0929	128.208± 1.3626	0.0074± 0.0015
Lycopene extract	5.5400± 0.5147	12.8066± 3.3111	9.4267± 0.8400	1.450± 3.9417	173.446± 1.8034	0.0128± 0.0130

The value represents mean and standard deviation (mean±standard deviation)

3.4 Stability of lycopene extracted from tomato

a) Effect of pH on the efficiency of lycopene colour

The tomato product is generally classified under acidic food as it contains approximately pH 4.6 for fresh tomato fruit. Result in Table 3.2 showed that the stability of lycopene pigment towards different pH values starting from acidic to alkaline. The percentage of remained and degraded lycopene was also tabulated in Table 3.2 below. The average mean of lycopene detected at 470 nm is ranging from 0.087 to 0.826 with standard deviation of 0.056 to 0.015 obtained after 30 minutes. The extracted

lycopene achieved the maximum stability at pH 5 with mean value 0.826 and percentage of degradation is only 18 %. This stability trend is followed by lycopene at pH 2 with average of 0.613 and 39 % degradation rate. Lycopene has been reported to have low stability in solutions that contain high acid amount. Akanbe and Oludemi [21] also reported that high acid content in tomato will promote to the higher lycopene degradation.

Based on the result, the lycopene was significantly affected by the alkaline medium where the highest percentage of degradation is noticed at pH 9 with 91.4 % of degradation rate. The mean of lycopene present at pH 7 and pH 12 are 0.192 and 0.316 with standard deviation of 0.018 and 0.020 respectively. According to Salama *et al.* (2015), it stated that beyond pH 4 value, a great loss of the pigment will be noticed especially at alkaline state (from pH 7 to pH 13). This result may be due to characteristic of double bonding system associated with carotenoid structure.

Data revealed that beyond pH 5, the lycopene content was reduced drastically and below pH 5 the lycopene are moderately affected by the solution after 30 minutes. The study from Vivek Kumar *et al.* [22] stated that the lycopene pigment is stable at ranged from pH 4.5 to 5.5. This statement is supported by Salama *et al.* [23] where the maximum stability of lycopene pigment can be achieved at pH 4 for 30 minutes. From the observation, it can conclude that the pH has significant effect on pigment intensity.

Table 3.2: The effect of pH on the percentage of degradation of lycopene pigment

pH value	Absorbance of lycopene at 470 nm	% remained of lycopene pigment (%)	% degradation of lycopene pigment (%)
2	0.613±0.013	61.0	39.0
5	0.826±0.015	82.0	18.0
7	0.192±0.018	19.0	81.0
9	0.087±0.056	8.6	91.4
12	0.316±0.020	31.2	68.8

The value represents mean and standard deviation (mean±standard deviation)

b) Effect of temperature on the efficiency of lycopene colour

The study from Evoli *et al.* [24] proved that thermal treatment can increase the bioavailability and extractability of lycopene pigment due to the disruption of protein complex inside the vegetable matrix. Therefore, lycopene also a heat sensitive compound and excessive heating might contribute to the pigment degradation due to the breaking of the protein-carotenoid complex that effects the stability of pigment. During thermal treatment, the heat released will induce the trans configuration to cis resulting in the modification of lycopene biological properties. Moreover, the exposure to light and oxygen will result in substantial degradation as heat disintegrate the tomato tissue during processing. In order to study this effect, the lycopene has been heated in the oven dryer at temperature ranged from 40 to 60 °C which resembled the cooking condition and the maximum temperature is lowered down as the maximum boiling point for n-hexane is 69 °C.

Table 3.3 shows the effect of temperature on the degradation rate of lycopene pigment at a certain temperature within 30 minutes. A slightly loss in stability of pigment was noticed at temperature 40 ℃ while it was increased at temperature 50 and 60 °C. Data from Table 3.3 shows that the mean value of lycopene pigment at 40, 50 and 60 °C is 0.824, 0.625 and 0.244 mg/kg respectively with standard deviation ranged from 0.047 to 0.198. The identification of lycopene was made by detection of UV-Vis spectra at 470 nm absorption band. At temperature 40 ℃, only 19 % of pigment are degraded compared to other temperatures which are 31 % and 76 % respectively. The high degradation rate of this pigment was noticed when heated at 60 °C. The percentage of pigment degradation is lower at

temperature 40 °C compared to temperature 50 and 60 °C. This is due to the increasing in the temperature that affects the instability within the lycopene pigment.

Some lycopene stability was also observed by Salama *et al.* [24] where the percentage of retention after the heat applied in ranged from 40 to 120 °C decreased by increasing the temperature. This indicates that at temperature plays the role for lycopene degradation. Takehara *et al.* [25] shows that 80 % of degradation rate have been detected in the solution after 10 hours as the thermal isomerization in hexane at 50 °C was conducted. This indicates that the isomerization can occur at 50 °C causing the 31 % reduction of lycopene in this experiment. The isomerization reaction mechanisms of all-trans isomers were reported as the lycopene pigment degrade with an average of the degradation rate is from 51.8 to 85.5 mg/100g in tomato at 50 °C [26].

Table 3.3: Effect of temperature on the degradation rate of lycopene pigment for 30 minutes.

Temperature/℃	Absorbance of lycopene at 470 nm	% remained of lycopene pigment (%)	% degradation of lycopene pigment (%)
40	0.824±0.047	81.0	19.0
50	0.652±0.012	69.0	31.0
60	0.244±0.198	24.0	76.0

The value represents mean and standard deviation (mean±standard deviation)

3.5 Antioxidant activity of lycopene pigment

Lycopene is a natural potent antioxidant due to the ability to act as a free radical scavenger. It is a reactive carotenoid in a biological system that depends on their molecular and physical structure, ability to interact with others antioxidant and concentration of oxygen. The percentage of antioxidant scavenging activity in lycopene pigment is calculated according to Gracia *et al.* [17]. DPPH radical scavenging assay has been used in order to conduct this test with ethanol as blank. The principle of this method is based on the removal of the stable DPPH radical from the reaction solution by the antioxidant action that is present in the sample. The mean value of antioxidant activity in lycopene extract is 66.350 % with standard deviation of 3.008 %. The data obtains in this study was quite low compare to others studies where the average percentage of scavenging activity recorded is in range of 72-94 % that are conducted in dark area for 30 minutes [27]. The radical scavenging activity data that was recorded by the Hussein *et al*. [28] is in range from 66.99 % to 90.12 % in tomato sample.

The main aspects that influence the reduction of antioxidant activity in this study are light intensity. The effect from light gives an impact on anti-radical activity measured during DPPH assay. Therefore, the high light intensity will promote the production of a compound that able to scavenge the peroxyl radical meanwhile, the low light intensity will produce the compound that inhibits lipid peroxidation. The highly conjugated double bond in lycopene plays an important role in energy transfer as the quenching ability towards singlet oxygen is based on excited energy state and length of this double bond [29]. Lycopene is the most efficient singlet oxygen quencher among the others carotenoid. The ability of this pigment to interact with ROS have prevented the tissue from cellular damage [30]. Excessive ROS level will cause damage to the DNA and proteins that lead to the apoptosis [31]. Therefore, lycopene exhibits antioxidant properties as it is important in scavenging of a reactive component.

3.6 Antimicrobial activity of lycopene pigment

Disk diffusion method has been used in this study as it's generally more sensitive and allowed quantitative determination of antibacterial efficiency. The lycopene extract was subjected to

antimicrobial activity against two bacterial strain which is gram-positive bacteria (*Bacillus cereus*) and gram-negative bacteria (*Serratia marcescens* SA30). The data is presented in Table 3.4 where the inhibition zone was recorded. The result is summarized as the lycopene extract exhibit higher antibacterial activity to the gram-positive bacteria than gram-negative bacteria. The mean values for inhibition zone of *Bacillus cereus* are 2.0 mm while there was no inhibition zone for *Serratia marcescens* SA30.

Table 3.4: Inhibition zone (mm) of the bacteria

Type of bacteria	Zone of inhibition (mm)
Bacillus cereus	2.0±3.4641
Serratia marcescens SA30	0

The value represents mean and standard deviation (mean±standard deviation)

Antibacterial activity from lycopene shows the best result towards the *Bacillus sp.* with the inhibition of 25 mm zone size [32]. Study from Kavitha *et al.* [33] also show the suppressive activity from lycopene toward *Bacillus subtillus* with the ranged of 2 to 9 mm of the zone of inhibition. Lycopene extract shows the antimicrobial properties to the *Bacillus cereus* in this experiment due to the presence of an active component in tomato on some bacteria [34]. Szabo *et al.* [35] reported that lycopene pigment extracted from various type of tomato presented a prone antimicrobial activity towards the gram-positive bacteria which is *Staphylococcus aureus* and *Bacillus subtills* with a range of minimum inhibitory concentration are 2.50 to 10 mg/ml than gram-negative strain. The interference of the lycopene with the cell wall biosynthesis of the *Bacillus sp.* will ultimately lead to the bacteria death [33]. Hence, when the lycopene sample added onto the bacterial lawn, they produce a clear diameter around the sample disk. In generally, the antimicrobial compound is more susceptible towards the gram-positive bacteria since they only have an outer peptidoglycan layer which is not an effective barrier for protection [36].

According to several journals, lycopene pigment shows less antibacterial activity against gram-negative bacteria. Therefore, a study from Szabo *et al.* [35] reported that the antimicrobial effectiveness from the tomato was affected by the types of cultivars. The *Taranesti roz* tomato cultivar shows the most efficient antimicrobial agent towards the gram-positive bacteria meanwhile *Abellus, Aphen* and *Cristal* varieties also show the significant effect against *E. coli* with MIC value with 5 mg tomato peels/ml. The present of beta-lactamases inhibitor has an impact on reducing the bacterial virulence on this strain. Thus, they conclude that the extract from tomato peel has acceptable antimicrobial activity against gram-positive and some varieties were effective towards gram-negative bacteria. A study from Dai *et al.* [30] state about the inhibition of gram-negative bacteria by using lycopene from tomato. From the research, they identified that lycopene can induce the ROS level and subsequent mitochondrial dysfunction. The result obtain in this test is relatively low compare to the previous study. A few factors that affect the accuracy and reproducibility of this result are the thickness and uniformity of the Muller-Hinton agar where the thickness for Kirby-Bauer method is commonly 4 mm thick [37].

4. Conclusion

In conclusion, the lycopene pigment was present in the *Solanum lycopersicon* extract due to the detection of spectrum at 470 and 471 nm by UV-Vis spectroscopy. The average concentration of lycopene obtained after extraction process using this method is 173.88 mg/kg. The lycopene pigment was susceptible to the degradation by pH and heat, as the reduction amount of lycopene was noticed after the pH and temperature test was conducted. This pigment shows the greatest stability at pH 5 and moderately degrade below this pH. Therefore, the lycopene is greatly affected at alkaline medium as during this study, 90 % of pigment degradation was recorded at pH 9 within 30 minutes. Thermal processing also gives an impact to the reduction of lycopene pigment in the sample. The lycopene loss

was noticed at temperature 50 and 60 °C in this study. The optimum percentage of antioxidant activity have been discovered in this extract which is 66.350 % but the value is lower compare to the others study as this sample have been exposed to the light during extraction process. The antimicrobial properties of lycopene are more susceptible to the gram-positive bacteria than gram-negative bacteria as no inhibition zone was recorded in *Serratia marcescens* SA30 during this study. An error has been identified due to the agar thickness for this test as it affecting the accuracy of the result and influences the agar coefficient diffusion.

Acknowledgement

The authors acknowledge with thanks to the Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia for technical and financial support.

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