Malaysian Journal of Analytical Sciences (MJAS)
Published by Malaysian Analytical Sciences Society

IDENTIFICATION AND QUANTIFICATION OF LYCOPENE AND β-CAROTENE IN WATERMELON JUICE USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

(Pengenalan dan Pengkuantitian Likopena dan B-Karotena di dalam Jus Tembikai Menggunakan Kromatografi Cecair Berprestasi Tinggi)

Nur Shafinaz Mohamad Salin[1], Fatimah Salim[2,3], Hairil Rashmizal Abdul Razak[4], Wan Mazlina Md Saad[1*]

[1]Centre of Medical Laboratory Technology, Faculty of Health Sciences
[2]Atta-ur-Rahman Institute for Natural Product Discovery (AuRIns)
Universiti Teknologi MARA Selangor Branch, Puncak Alam Campus, 42300 Puncak Alam, Selangor, Malaysia.
[3]Centre of Foundation Studies,
Universiti Teknologi MARA Selangor Branch, Dengkil Campus, 43800 Dengkil, Selangor, Malaysia.
[4]Department of Radiology, Faculty of Medicine and Health Sciences,
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

**Corresponding author: wanmaz755@uitm.edu.my*

Received: 18 October 2021; Accepted: 21 November 2021; Published: 27 December 2021

Watermelon (*Citrullus lanatus*) is a nutritional fruit with appealing flesh colour. The flesh colour is reflected by the presence of carotenoid compounds, lycopene and β-carotene. Lycopene is responsible for the deep red fruit colour while β-carotene provides the orange-yellow pigment. In addition, these carotenoid components can scavenge free radicals and protect one from harmful diseases. In this study, isocratic mode high-performance liquid chromatography (HPLC) was performed to separate and quantify lycopene and β-carotene in watermelon juice. Twenty microliter sample injection was passed through C-18 column maintained at 45 °C coupled with diode array detector (DAD) at 470 nm. Mobile phase of acetonitrile and water (95:5, v/v) were at the flow rate of 1 mL/min. Excellent chromatographic separation of lycopene and β-carotene were achieved at elution time of 4.568 and 6.831 min, respectively. The amount of quantified lycopene (1662 µg/mL) and β-carotene (180 µg/mL) indicated that lycopene is the major carotenoid present in watermelon juice. This study described the ability of isocratic mode HPLC to separate and quantify carotenoid in watermelon juice. The analytical method had been validated, with the results showing precision, accuracy, and linearity.

Keywords: watermelon, carotenoid, lycopene, β-carotene, high-performance liquid chromatography

Abstrak

Tembikai (*Citrullus lanatus*) ialah buah bernutrisi yang mempunyai warna isi yang menarik. Warna isi buah tembikai ini dicerminkan oleh kandungan sebatian karotenoid; likopena dan β-karotena. Sebatian karotenoid ini bertanggungjawab sebagai pigment warna pada buah. Tambahan pula, komponen karotenoid ini dapat menghalang serangan radikal bebas serta melindung kita daripada pelbagai jenis penyakit berbahaya. Dalam kajian ini, kaedah isokratik kromatografi cecair berprestasi tinggi (KCPT) dilakukan untuk memisahkan dan mengkuantitikan kandungan likopena dan β-karotena di dalam jus tembikai. Muatan

sampel 20 µL dilalukan kedalam turus C-18 pada suhu dikekalkan 45 °C ditambah dengan pengesan susan diod (PSD) pada 470 nm. Gabungan pelarut asetonitril dan air (95:5, v/v) dengan kadar aliran 1 mL/min telah digunakan. Pemisahan kromatografi likopena dan β-karotena yang sangat baik telah dicapai pada masa elusi 4.568 dan 6.831 minit. Jumlah pengkuantitian likopena ialah 1662 µg/mL dan β-karotena 180 µg/mL, menunjukkan bahawa likopena ialah karotenoid utama diikuti dengan β-karotena di dalam jus tembikai. Dapatan kajian ini menerangkan kemampuan KCPT mod isokratik untuk mengenalpasti dan mengkuantitikan profil karotenoid di dalam jus tembikai. Kaedah analitikal yang digunakan bagi kajian ini telah divalidasi dan dapatan kajian menunjukkan kejituan, ketepatan dan kelinearan.

Kata kunci: tembikai, karotenoid, likopene, β-karoten, kromatografi cecair berprestasi tinggi

Introduction

Consumers are increasingly showing greater interest in the consumption of fruits that positively impact health and well-being. Besides the physical appearance of colour, taste and aroma, the presence of bioactive compounds including carotenoids, flavonoids, phenolics and vitamins are also prominent in fruits. Watermelon, scientifically known as *Citrullus lanatus,* belongs to the Cucurbitaceae family [1] and is non-seasonal fruit that has been cultivated abundantly in Malaysia and other tropical regions [2]. Watermelon constitutes 93% water, hence the name 'water'. 'Melon' refers to the fruit's morphological characteristics that are round or oval, sweet and pulpy fleshed [2]. Watermelon is also referred to as 'pepo' by botanists, referring to its thick rind and fleshy centre [3]. The fruit is categorised as having three different parts that include flesh (68%), peel (30%), and seed (2%). Watermelon flesh mainly comprises water content [4] and is reported to be a rich source of bioactive compounds such as lycopene, β-carotene, L-citrulline and vitamins (A and C) [5].

Watermelons come in a variation of flesh colours of red, yellow, orange or white, and are either seeded or seedless [6]. The flesh colour is an essential trait of watermelon fruit [7] and the appealing flesh colour reflects the total carotenoid compound present. Carotenoid belongs to the tetraterpenes family and are sources of red, orange and yellow colour pigmentations in fruits [8]. Red watermelon was reported to have major lycopene content followed by secondary carotenoid compound that include β-carotene [5]. Lycopene and β-carotene are responsible for the deep red and orange-yellow pigments respectively [9]. More importantly, watermelon has been reported to have a large lycopene content compared to other fruits [4]. Moreover, study by Zhang et al. further reported that watermelon contains high amount of lycopene with 50 mg/100g compared to tomato with only 30 mg/100g [10]. Furthermore, lycopene and β-carotene have been well studied as antioxidants that able to quell free radicals and oxidative damage [9,11]. In addition, the daily consumption of lycopene and β-carotene have been found to significantly promote beneficial health effects [4]. The lycopene content in fruits can reduce oxidative damage resulting in chronic diseases [12], inhibit tumor development in liver, lung, prostate, breast and colon [9] and prevent the pathogenesis of Alzheimer's disease [4]. Moreover, the secondary carotenoid, β-carotene, exhibits antioxidant, anticancer and anti-inflammation properties that are beneficial to health. It has also been proven to be a pro Vitamin A precursor and promotes eye health [11,13]. Considering the nutritional and sensorial functions of lycopene and β-carotene in watermelon, researchers concerned with the development of functional food have now focused on watermelon as a valuable source of carotenoid and its positive health effects.

Various methods have been performed to identify and quantify carotenoid compounds in fruit. The main methods used are colorimetric and chromatographic analysis such as high-performance liquid chromatography (HPLC), liquid chromatography mass spectrometer (LCMS), spectrophotometry, supercritical fluid chromatography and comprehensive two-dimensional liquid chromatography [14]. However, HPLC is the preferred method to quantify carotenoid due to its sensitivity and specificity with ideal column and detector, efficiency in saving time and minimal sample usage [14,15]. Table 1 tabulated the

analytical chromatographic method for lycopene and β-carotene quantification using HPLC. Studies had reported the used of C-30 carotenoid YMC column coupled with photo-diode array (PDA) [16] and C-18 column paired with UV-visible detector (UV-vis) able to separate carotenoid in fruit [17]. On the other hand, a study by Petyaev et al. reported the use of C-18 column coupled with photo diode array detector (PAD) in quantification of carotenoid using HPLC [18]. However, a previous chromatographic analysis was in gradient mode [16], with more than two mobile phase [16,17,18] and the results showed that carotenoid was determined at 12-15 minutes [4,16,17]. Hence, the development of fast and minimal usage of mobile phase are desirable. In this study, high-performance liquid chromatography (HPLC) was chosen along with C-18 column and diode array detector (DAD) with isocratic mobile phase of acetonitrile: water (95:5, v/v) to identify and quantify lycopene and β-carotene in watermelon (*Citrullus lanatus*) juice.

Materials and Methods

Chemicals and reagents

Analytical grade of lycopene (≥98%) and β-carotene (≥95%) were purchased from Sigma Aldrich (St. Louis, MO, USA). HPLC grade of n-hexane, tetrahydrofuran, methanol (MeOH) and acetonitrile (MeCN) were purchased from Merck (Germany).

Instruments

High-performance liquid chromatography (HPLC) was performed using Agilent 1200 (Agilent Technology, 1200 Series) equipped with auto-sampler injector (G1328B), column oven, quat pump (G1311A), degasser (G1311A) and diode array detector (DAD) for separation of lycopene and β-carotene. The column used was ZORBAX (Agilent) 300 SB-C18 column, 5 µm (4.6 x 150 mm).

Watermelon juice preparation

Six watermelons (*Citrullus lanatus)* ranging from 2.2 to 3.2 kg were obtained from a local farm located in Subang, Selangor, Malaysia. The sample species were identified by Forest Research Institute Malaysia (FRIM) with reference no: FRIM700-1/1/1Kit.3(87). The watermelons were washed and wiped with paper towels. Watermelons were then peeled, weighed and cut before homogenized into juice. The obtained watermelon juice with volume of 100 mL were then extracted with 100 mL of n-hexane for 24 hours on a reciprocating shaker (180 rpm). After 24 hours, the watermelon juice was aliquot into 50 mL of capped falcon tube and later centrifuged at 3000 rpm for 10 minutes. The centrifugation resulted in three separated layers of watermelon juice consisting yellowish-orange hexane supernatant (upper), turbid and white colour (middle) and red watermelon flesh residue (bottom). The obtained yellowish supernatant containing lycopene and β-carotene were later separated and evaporated to dryness using rotary evaporator to obtain the watermelon extract. The n-hexane separated layer was later removed. Meanwhile, the residue of the middle and bottom juice layers was twice subjected to the extraction step with n-hexane to completely extract the carotenoid compounds. The watermelon juice extract was then stored in -80 °C prior analysis. All procedures were performed in the dark with minimal light and air exposure to reduce degradation of targeted carotenoids.

Standards preparation

One milligram of each lycopene and β-carotene standard were dissolved in tetrahydrofuran (0.5 mL) and methanol (0.5 mL) to obtain stock solution of 1000 µg/mL and later filtered through 0.45-µm polyvinylidene fluoride (PVDF) syringe filter into 2 mL of amber HPLC vial. A series of working solutions for each standard were prepared by diluting the stock solution two-folds in ranges 31.25-1000 µg/mL.

Sample preparation procedure

Approximately 500 µg/mL of extract sample was prepared by dissolving 0.5 mg of watermelon juice extract in 1 mL of tetrahydrofuran and methanol (1:1). The extract solution then was filtered through 0.45-µm PVDF syringe filter into 2 mL of amber HPLC vial to reduce light exposure.

Isocratic mode of high-performance liquid chromatography-diode array detector

Identification and quantification of lycopene and β-carotene were performed referring to Noh et al. with

slight modifications [4]. The separation was conducted by injecting 20 µL of each standard and sample into ZORBAX SB-C18 column, 5 µm (4.6 x 150 mm), at 45 °C using isocratic mode of HPLC mobile phase MeCN and water (95:5, v/v) with flow rate of 1 mL/min. The separation and detection of lycopene and β-carotene in standard and sample were carried out triplicate into C-18 column coupled with diode array detector at wavelength 470 nm.

Method validation

The validation method for identification of carotenoid using isocratic mode HPLC was achieved by determining the linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy and recovery according to the International Conference of Harmonization [19]. The linearity was determined by injecting 20 µL of five different concentrations of each standard at ranging 31.25-1000 µg/mL into HPLC column. A linear regression analysis was constructed by plotting a graph peak area against standard concentrations. The regression equation was then calculated in the form of y = a+bx, where x is the concentration while y is the peak area of the targeted compound. Linearity was assessed by the expression of regression coefficient (R^2) value.

LOD is the lowest amount of compound that could be detected while LOQ is the lowest quantified amount of interest analyte. The LOD and LOQ were expressed as 3 Sa/b and 10 Sa/b, where Sa is the standard deviation of response and b is the slope of calibration curve [1,2]. Triplicate spike concentration of lycopene and β-carotene at 300, 150 and 100 µg/mL were injected to test sample, and the recovery was calculated with the value of detected versus added amounts. Precision of method used was caried out by determining the percentage relative standard deviation (%RSD) and recovery (%R) of peak area for inter-day and intra-day analysis. The analysis for inter-day and intra-day were performed at three different concentrations of each standard (500, 300, 150 µg/mL), as referred by Rasdin et al. [1].

Table 1. Chromatographic analytical method in quantification of carotenoid using HPLC

Column	Detector	Mode	Mobile Phase	References
C-18	Diode array	Isocratic	100% acetonitrile	[4]
C30 YMC	UV-Vis	Isocratic	Methanol: isopropyl alcohol: tetrahydrofuran (30:30:35, v/v/v) containing 250 ppm butylated hydroxytoluene and 0.5% tryethylamine	[14]
C30 YMC Carotenoid S-5	Photo-diode array	Gradient	Acetonitrile-methanol and methyl tert-butyl ethanol	[16]
C-18	UV-Vis	Isocratic	Methanol: tetrahydrofuran: water (60:33:7, v/v)	[17]
Aquity HSS T3	Photo diode array	Isocratic	Acetonitrile:0.08% phosphoric acid solution : tert-butyl methyl ether (70:5:25, v/v/v)	[18]

Results and Discussion

Identification and quantification of lycopene and β-carotene in watermelon juice extract

This study demonstrated the identification and quantification of lycopene and β-carotene in watermelon juice using isocratic mode in which HPLC was paired with C-18 column and diode array detector (DAD). Figure 1 shows the chromatogram of standard lycopene (A), β-carotene (B) and watermelon juice (C). The chromatogram's peak of watermelon juice was determined by comparing the retention time of standard lycopene and β-carotene. To produce an optimum separation of lycopene and β-carotene in the standard and sample, the chromatographic method from Noh et al. was adapted with slight modifications [4]. Triplicate injection (20 µL) of each standard and sample were injected through short C-18 column at 45 °C coupled with diode array detector at 470 nm that showed the best separation and detection of targeted lycopene and β-carotene with noise eliminated. Column type and temperature used strongly influenced the separation of interest analyte.

In previous studies, C-18 column was used with various temperature tested for separation of lycopene and β-carotene using HPLC [16, 23]. This study found that ZORBAX SB-C18 column, 5 µm (4.6 x 150 mm) was able to operate and separate efficiently at temperature 45 °C. Hence, the column temperature was set at 45 °C. In addition, lycopene and β-carotene are tetraterpenes comprising eight isoprene units that form a conjugated double bond solely composed of carbon and hydrogen. The double bond structure acts as chromophore that allows the absorption of light ranges 420 - 520 nm [4]. Hence, the detection wavelength of 470 nm was chosen as the lycopene and β-carotene appears to absorb the most at this wavelength with no other peak eluted in the chromatogram [4, 20].

An isocratic mode of mobile phase MeCN: water (95:5, v/v) with flow rate of 1 mL/min showed the optimum ratio composition for producing high selectivity and resolution peak of targeted lycopene and β-carotene using HPLC. This agrees with the study performed by Noh et al. using isocratic mode HPLC with 100% acetonitrile as a mobile phase in chromatographic analysis [4]. Carotenoid is a non-polar compound. Thus, reversed-phase HPLC is preferable to identify lycopene and β-carotene in samples. Acetonitrile with high polarity property has been reported to be able to detect carotenoid in fruits, and have been employed by other researchers [4, 20]. However, the addition of 5% water into HPLC mobile phase could elute lycopene faster compared to previous study [4]. Hence, this study has shown that the mobile phase of acetonitrile and water with ratio 95:5 is able to identify lycopene and β-carotene selectively.

Figure 1 above presents a good separation of lycopene and β-carotene in standard and sample of watermelon juice extract. The elution peak sequence of lycopene (4.568 min) and β-carotene (6.831 min) were in line with the study carried out by Jin et al. [21]. In the watermelon juice extract, lycopene was detected at retention time of 4.549 min while β-carotene was eluted at 6.790 min, respectively. Studies have reported that lycopene is more polar than β-carotene [22]. Thus, lycopene peak was eluted first followed by β-carotene due to its different polarity properties. This study showed that lycopene and β-carotene could be separated and identified using isocratic mode HPLC coupled with selective column, detector and mobile phase chosen.

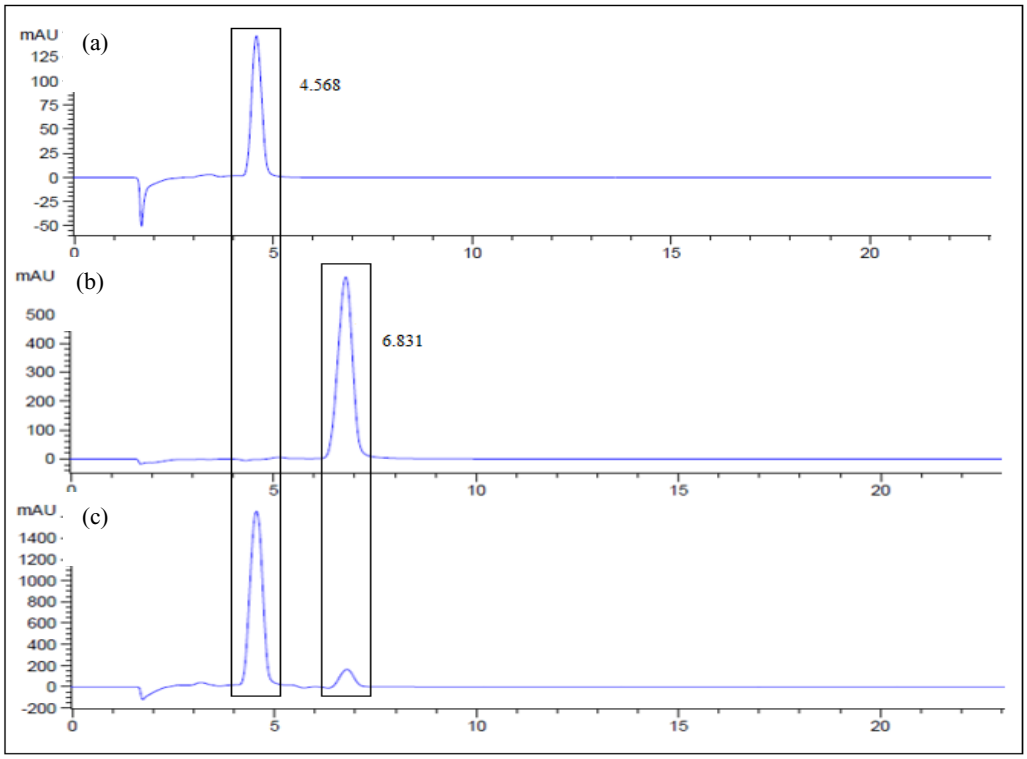

Figure 1. The chromatogram of standards and sample (a) Standard of lycopene at 4.568 minutes, (b) Standard of β-carotene at 6.831 minutes and (c) Watermelon juice of lycopene and β-carotene at retention time of 4.549 and 6.790 minutes

Method validation

In this study, optimized chromatographic analysis was validated using linearity, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy as referred to International Conference of Harmonization [19]. Table 2. shows the validation method for regression equation, LOD and LOQ of lycopene and β-carotene. The regression equation for lycopene and β-carotene were y= 1.2029 x-6.5599 and y= 5.8788 x-437.57 respectively. Correlation coefficient (R^2) for both lycopene and β-carotene were at 0.9972 and 0.995, respectively (Table 2). The R^2 value was compared to previous studies that demonstrated the R^2 of carotenoid ranges of 0.9931-0.9993 [4,17]. Moreover, a study by Zhang et al. (2016) further supported the R^2 value ≥0.995 which indicated that the presented data was linear and reproducible [23]. LOD is the lowest amount of interest analyte detected while LOQ is the lowest amount of targeted compound quantified in the sample [1]. The presented LOD and LOQ values for both lycopene and β-carotene were in the ranges of 0.302 - 10.09 µg/mL (Table 2). The LOD and LOQ values were compared to Noh et al (2020) which showed that the present study was accurate and sensitive for the detection of carotenoid in watermelon juice extract [4].

In Table 3, the recovery data for spike and measured carotenoid were tabulated. The data was obtained by spiking triplicate 300, 150 and 100 µg/mL standard of lycopene and β-carotene into the test sample. The recovery for spike and measured concentration were found to be in the ranges of 99.47-109.49 %, respectively, well within the range reported in previous study [24]. The expression of regression standard deviation (%RSD) between the mean of analysed compound for inter-day and intra-day analysis were tabulated in Table 4. Triplicate injection of 500, 300

and 150 µg/mL of each standard were analysed for its validity. The RSD values for intra-day and inter-day analysis were ranges of 1.4-8.1% while the percentage recovery was found to be in the ranges of 92-113% respectively (Table 4). This result of this study is in line with Gebregziabher et al. that demonstrated the recovery for lycopene and β-carotene as more than 83.12% [24]. Besides, the RSD values were below than 10% as supported by Zhang et al. [23]. This indicated that the proposed method could be used for simultaneous determination of lycopene and β-carotene in watermelon juice [7]. Hence, the validation results of linearity, LOD, LOQ, accuracy and precision values showed that the isocratic mode of HPLC used in this study was sensitive and precise in the separation and detection of lycopene and β-carotene in watermelon juice extract.

Table 2. The validation method for lycopene and β-carotene

Standard	Regression Equation	Correlation Coefficient (R^2)	Linear Range (µg/mL)	Limit of Detection (LOD) (µg/mL)	Limit of Quantification (LOQ) (µg/mL)
Lycopene	y = 1.2029 x - 6.5599	0.9972	31.25-1000	3.33	10.09
β-carotene	y = 5.8788 x - 437.57	0.9955	31.25-1000	0.995	0.302

Table 3. Recovery data for spike and measured lycopene and β-carotene in watermelon juice

Compound	Spike Concentration (µg/mL)	Measured Concentration (µg/mL)	Recovery (%)
Lycopene	300	301.05	100.18
	150	164.24	109.49
	100	102.31	102.31
β-carotene	300	296.81	99.47
	150	149.72	99.81
	100	102.54	102.54

Table 4. Intra-day and inter-day data for lycopene and β-carotene standard

Compound	Concentration (µg/mL)	Intra-day n=3 (%)			Inter-day n=3 (%)		
		Mean	RSD	Recovery	Mean	RSD	Recovery
Lycopene	500	495.23	7.7	99	520.64	6.1	104
	300	300.59	1.4	100	286.14	5.0	95
	150	143.07	6.2	95	160.72	4.3	107
β-carotene	500	468.58	2.2	93	461.69	8.1	92
	300	280.94	4.4	94	285.00	7.4	95
	150	160.500	6.0	106	169.50	5.2	113

Quantification of lycopene and β-carotene

The consumption of watermelon juice naturally enriched with carotenoid compounds such as lycopene and β-carotene could eliminate free radicals and benefit health. Thus, an efficient and simple isocratic mode of HPLC is essential in the quantification of carotenoid compounds in fruits. The concentration of lycopene and β-carotene in watermelon juice were quantified using the regression equation obtained from calibration curve. The quantification of lycopene and β-carotene in watermelon juice were tabulated in Table 5. The quantification showed that lycopene is the major carotenoid compound with 1662 µg/mL followed by β-carotene with 180 µg/mL. This study agrees with the study by Tamburini et al. which concurred that watermelon juice has the highest lycopene concentration of 2.65–151.75 mg/kg while β-carotene is only 0.19–9.39 mg/kg [5]. It is documented that watermelon contributes the highest lycopene concentration, 40% of total carotenoid present in fruits [25]. It is also supported by Zhao et al. that watermelon provides approximately 84-97% lycopene, while β-carotene, only 2-11% [26]. Hence, increased lycopene concentration indicated increased the red intensity in fruits. This study concurs that the high content of lycopene gives an appealing and attractive red intensity in watermelon flesh.

Besides colour pigmentation, increased lycopene concentration had been reported to promote antioxidant activities. Lycopene is the strongest antioxidant among carotenoids [12, 27]. This antioxidant property is capable of quenching free radicals and preventing the development of reactive oxygen species (ROS) that may adversely affect health conditions [27]. Besides, lycopene has also been reported as anti-inflammation, anti-aggregative, anti-hypertensive, anti-atherosclerotic, and acts as a cardioprotective agent [28]. Ghadage et al. demonstrated that the consumption of diet enriched with lycopene was associated with decreased risk of chronic diseases including cardiovascular disease and cancer [29]. Thakur also supported that lycopene acted as inhibitor for tumour development in liver, lung, prostate and colon [9]. Hence, the present quantitative result confirmed that lycopene and β-carotene could be quantified in watermelon juice extract using isocratic mode HPLC. Thus, the study suggests that the consumption of watermelon juice enriched with lycopene is essential in prevention of health diseases.

Table 5. Concentration of lycopene and β-carotene in watermelon juice extract

Compound	Concentration in watermelon juice extract (µg/mL)
Lycopene	1662
β-carotene	180

Conclusion

The HPLC analysis successfully identified and quantified respective carotenoids; lycopene and β-carotene in watermelon juice. The study results showed that this method is sensitive and reproducible with linearity (≥0.995), low LOD and LOQ values in ranges of 0.30-10.09 µg/mL with recovery 99.47-109.49 % as well as RSD less than 10%. The study presented that watermelon juice contains high amount of lycopene (1662 µg/mL) compared to β-carotene (180 µg/mL) which lends a high red intensity in flesh colour. This study also serves as a base reference for future research to quantify carotenoid compound using simple chromatographic analysis. However, further study is needed to complete carotenoid profiling and quantification in watermelon juice using isocratic mode

HPLC. It is also necessary to explore the roles of these carotenoid properties in watermelon as a nutritional fruit in maintaining a healthy lifestyle.

Acknowledgement

The authors would like to acknowledge (1) Ministry of Higher Education, Malaysia, through Research Management Centre, Universiti Teknologi MARA (UiTM) for funding the study (RMC grant no. 600-RMC/GPK 5/3 (074/2020)); (2) Centre of Postgraduate Study, Faculty of Health Sciences, Universiti Teknologi MARA (UiTM), Puncak Alam Campus, and Analytical Unit Laboratory, Faculty of Pharmacy, Universiti Teknologi MARA (UiTM), Puncak Alam Campus for providing the research facilities and work place throughout this study; (3) Local farm in Subang, Selangor, Malaysia for providing watermelon samples.

References

1. Ridwan, R., Abdul Razak, H. R., Adenan, M. I. and Md Saad, W. M. (2018). Development of isocratic RP-HPLC method for separation and quantification of L-citrulline and L-arginine in watermelons. *International Journal of Analytical Chemistry*, 2018: 4798530.
2. Saad, W. M. M., Salin, N. S. M., Ramzi, A. S. and Salim, F. (2020). Identification and quantification of fructose, glucose and sucrose in watermelon peel juice. *Malaysian Journal of Analytical Sciences*, 24(3): 382-389.
3. Maoto, M. M., Beswa, D. and Jideani, A. I. (2019). Watermelon as a potential fruit snack. *International Journal of Food Properties*, 22(1): 355-370.
4. Noh, J. J., Hur, O. S., Ro, N. Y., Lee, J. E., Hwang, A. J., Kim, B. S., Rhe, J. S., Yi, J. Y., Kim, J. H., Lee, H. S., Sung, J. S., Kim, M. K. and Assefa, A. D. (2020). Lycopene content and fruit morphology of red, pink, orange, and yellow fleshed watermelon (*Citrullus lanatus*) germplasm collections. *Korean Journal of Plant Resources*, 33(6): 624-637.
5. Tamburini, E., Costa, S., Rugiero, I., Pedrini, P. and Marchetti, M. G. (2017). Quantification of lycopene, β-carotene, and total soluble solids in intact red-flesh watermelon (*Citrullus lanatus*) using on-line near-infrared spectroscopy. *Sensors*, 17(4): 746.
6. Kyriacou, M. C., Leskovar, D. I., Colla, G. and Rouphael, Y. (2018). Watermelon and melon fruit quality: The genotypic and agro-environmental factors implicated. *Scientia Horticulturae*, 234: 393-408.
7. Zhang, L., Wang, S., Yang, R., Mao, J., Jiang, J., Wang, X., Zhang, W., Zhang, Q. and Li, P. (2019). Simultaneous determination of tocopherols, carotenoids and phytosterols in edible vegetable oil by ultrasound-assisted saponification, LLE and LC-MS/MS. *Food Chemistry*, 289: 313-319.
8. Prakash, D. and Gupta, C. (2014). 12 Carotenoids: chemistry and health benefits. Phytochemicals of Nutraceutical Importance, CABI International Publishers, Wallingford (2014), pp. 181-195.
9. Thakur, N. (2016). Lycopene quantification of tomato by SPE and HPLC. *Bulgarian Journal of Agricultural Science*, 22(1): 84-90.
10. Zhang, J., Sun, H., Guo, S., Ren, Y., Li, M., Wang, J., Zhang, H., Gong, G. and Xu, Y. (2020). Decreased protein abundance of lycopene β-cyclase contributes to red flesh in domesticated watermelon. *Plant Physiology*, 183(3): 1171-1183.
11. Bogacz-Radomska, L. and Harasym, J. (2018). β-Carotene-properties and production methods. *Food Quality and Safety*, 2(2): 69-74.
12. Karahan, F., Dede, S. and Ceylan, E. (2018). The effect of lycopene treatment on oxidative DNA damage of experimental diabetic rats. *The Open Clinical Biochemistry Journal*, 8(1):1-6.
13. Milani, A., Basirnejad, M., Shahbazi, S. and Bolhassani, A. (2017). Carotenoids: biochemistry, pharmacology and treatment. *British Journal of Pharmacology*, 174(11): 1290-1324.
14. Cucu, T., Huvaere, K., Van Den Bergh, M. A., Vinkx, C. and Van Loco, J. (2012). A simple and fast HPLC method to determine lycopene in foods. *Food Analytical Methods*, 5(5): 1221-1228.
15. Adadi, P., Barakova, N. V. and Krivoshapkina, E. F. (2018). Selected methods of extracting carotenoids, characterization, and health concerns: A review. *Journal of Agricultural and Food Chemistry*, 66(24): 5925-5947.

16. Huang, J. and Hui, B. (2020). The determination of lycopene Z-isomer absorption coefficient on C30-HPLC. *Food Science & Nutrition*, 8(11): 5943-5952.
17. Sirikhet, J., Chanmahasathien, W., Raiwa, A. and Kiattisin, K. (2021). Stability enhancement of lycopene in *Citrullus lanatus* extract via nanostructured lipid carriers. *Food Science & Nutrition*, *9*(3): 1750-1760.
18. Petyaev, I. M., Pristensky, D. V., Morgunova, E. Y., Zigangirova, N. A., Tsibezov, V. V., Chalyk, N. E. and Bashmakov, Y. K. (2019). Lycopene presence in facial skin corneocytes and sebum and its association with circulating lycopene isomer profile: Effects of age and dietary supplementation. *Food Science & Nutrition*, 7(4): 1157-1165.
19. International Conference on Harmonization (2003). Technical requirements for the registration of pharmaceuticals for human use, validation of analytical procedures: Methodology. The 6th International Conference on Harmonization, Geneva, 2003
20. Pataro, G., Carullo, D., Siddique, M. A. B., Falcone, M., Donsì, F. and Ferrari, G. (2018). Improved extractability of carotenoids from tomato peels as side benefits of PEF treatment of tomato fruit for more energy-efficient steam-assisted peeling. *Journal of Food Engineering*, 233: 65-73.
21. Jin, H., Lao, Y. M., Zhou, J., Zhang, H. J. and Cai, Z. H. (2017). Simultaneous determination of 13 carotenoids by a simple C18 column-based ultra-high-pressure liquid chromatography method for carotenoid profiling in the astaxanthin-accumulating *Haematococcus pluvialis*. *Journal of Chromatography A*, 1488: 93-103.
22. Kehili, M., Kammlott, M., Choura, S., Zammel, A., Zetzl, C., Smirnova, I. and Sayadi, S. (2017). Supercritical CO_2 extraction and antioxidant activity of lycopene and β-carotene-enriched oleoresin from tomato (*Lycopersicum esculentum* L.) peels by-product of a Tunisian industry. *Food and Bioproducts Processing*, 102: 340-349.
23. Zhang, C-R., Aldosari, S. A., Vidyasagar, P. S., Shukla, P. and Nair, M. G. (2016). Determination of the variability of sugars in date fruit varieties. *Journal of Plantation Crops*, 43(1): 53-61.
24. Gebregziabher, B. S., Zhang, S., Qi, J., Azam, M., Ghosh, S., Feng, Y. and Sun, J. (2021). Simultaneous determination of carotenoids and chlorophylls by the HPLC-UV-Vis method in soybean seeds. *Agronomy*, 11(4): 758.
25. United States Department of Agriculture (USDA). (2002). Watermelon packs a powerful lycopene punch. Retrieved from https://agresearchmag.ars.usda.gov/2002/jun/.
26. Zhao, W., Lv, P. and Gu, H. (2013). Studies on carotenoids in watermelon flesh. *Agricultural Science*, 4(7): 8.
27. Przybylska, S. (2020). Lycopene a bioactive carotenoid offering multiple health benefits: A review. *International Journal of Food Science & Technology*, 55(1), 11-32.
28. Mozos, I., Stoian, D., Caraba, A., Malainer, C., Horbańczuk, J. O. and Atanasov, A. G. (2018). Lycopene and vascular health. *Frontiers in Pharmacology*, 9: 521.
29. Ghadage, S. R., Mane, K. A., Agrawal, R. S. and Pawar, V. N. (2019). Tomato lycopene: Potential health benefits. *The Pharma Innovation Journal*, 8(6): 1245-1248.