Comparative analysis of carotenoid content in *Momordica cochinchinensis* (Cucurbitaceae) collected from Australia, Thailand and Vietnam

Dilani Wimalasiri¹ · Robert Brkljač³ · Terrence J. Piva² · Sylvia Urban³ · Tien Huynh¹

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Abstract *Momordica cochinchinensis* (Cucurbitaceae) is the richest source of lycopene and β -carotene of all known fruits but the influences of collection sites, variety and environment on carotenoid accumulation is unknown. This study analysed the carotenoid content of 44 *M. cochinchinensis* aril samples collected from Australia, Thailand and Vietnam using HPLC, UV–visible spectrophotometry and compared with the colorimetry method. The highest lycopene content was observed in samples collected from Ha Noi (7.76 mg/g) of Northern Vietnam and Lam Ha (6.45 mg/g) and Lam Dong (6.64 mg/g) provinces of Central Vietnam. The highest β -carotene content was observed in a sample from Nam Dinh (9.60 mg/g) in Northern Vietnam while a variety from Hoa Binh province in Northern Vietnam had high contents of both lycopene (5.17 mg/g) and β -carotene (5.66 mg/g). Lycopene content was higher in samples collected from low temperatures ($<14^{\circ}\text{C}$) and higher elevations whilst β -carotene content was greatest at temperatures between 27 and 33°C . Crop improvement for increased lycopene and

β -carotene requires rapid and accurate methods of quantification. All three analytical methods utilised were in agreement for lycopene quantification. The $(a^*/b^*)^2$ transformed colour value resulted in more linear relationship for lycopene indicating that colorimetry method could potentially be developed to select lycopene rich fruits in the field.

Keywords *Momordica cochinchinensis* · Collection sites · β -carotene · High performance liquid chromatography (HPLC) · Lycopene

Introduction

Carotenoids are fat soluble pigments synthesized in plants, photosynthetic bacteria and fungi through the mevalonic acid pathway using an isopentenyl pyrophosphate (IPP) precursor (Rodriguez-Amaya and Kimura 2004). The main carotenoids are lycopene and β -carotene, which are the compounds responsible for the red and yellow-orange colour, respectively. Both lycopene and β -carotene have numerous bio-medicinal and nutraceutical benefits (Rao and Rao 2007). Both pigments are highly effective antioxidants due to their conjugated double bond system and free radical scavenging activity. Amongst carotenoids, lycopene has the highest singlet oxygen-quenching rate (Perkins-Weazie et al. 2001) and is responsible for the reduction of cancers, cardiovascular diseases and macular dysfunction (Rao and Rao 2007). Lycopene is the precursor of most cyclic carotenoids including β -carotene, which converts to produce vitamin A, important for the development of cell membranes, immunity and skin health (Hammond and Renzi 2013; Rodriguez-Amaya and Kimura 2004).

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✉ Tien Huynh
tien.huynh@rmit.edu.au

¹ School of Applied Sciences (Biotechnology), RMIT University, PO Box 71, Bundoora 3083, Australia

² School of Medical Sciences, Health Innovations Research Institute (HIRi), RMIT University, PO Box 71, Bundoora 3083, Australia

³ School of Applied Sciences (Chemistry), Health Innovations Research Institute (HIRi), RMIT University, GPO Box 2476V, Melbourne, VIC 3001, Australia

Momordica cochinchinensis based products have potential in the nutraceutical and functional food industries due the high carotenoid concentration in this fruit (Supplementary Fig. 1). Currently, the commonly recognised food source of lycopene is tomatoes and for β -carotene it is carrots. *M. cochinchinensis* have the highest content of dietary β -carotene and lycopene, with contents 10–70 fold more than all known fruits and vegetables (Aoki et al. 2002) including tomatoes (8.8–42.0 $\mu\text{g/g}$ FW lycopene) (Rao and Rao 2007) and carrots (47–11,210 $\mu\text{g/g}$ FW β -carotene) (Gul et al. 2015). Despite its superior carotenoid content and phytochemical potential (Chuyen et al. 2015), *M. cochinchinensis* is genetically diverse and geographically restricted (Bootprom et al. 2012; Wimalasiri et al. 2016); it is not widely distributed outside of South East Asia (Aoki et al. 2002). There is limited information on varietal differences and the downstream effects on carotenoid accumulation, which hinder the selection of breeding stock.

Carotenogenesis in other economically important fruits, including apricots (Dragovic-Uzelac et al. 2007), carrots (Leja et al. 2013) and tomatoes, has been shown to be influenced by geographical and environmental factors, including elevation (Rodriguez-Amaya and Kimura 2004), rainfall (Brandt et al. 2003) and temperature (Leoni 1992; Dumas et al. 2003), with carotenoid synthesis impacted more by geographical location than varietal differences (Aherne et al. 2009) or vice versa (Leja et al. 2013). *M. cochinchinensis* has a diverse distribution in South East Asia from tropical to temperate regions with varying eco-geographical profiles (Wimalasiri et al. 2016), which could account for the nutritional variations previously reported (Chuyen et al. 2015). Cultivar selection and optimal growth conditions are essential for producing consistently higher quantities of carotenoids but in the case of *M. cochinchinensis* these parameters are unknown.

Development of cultivars with higher carotenoid content using traditional breeding approaches requires rapid screening methods. The carotenoids in *M. cochinchinensis* are commonly quantified using High Performance Liquid Chromatography (HPLC) (Ishida et al. 2004; Kubola and Siriamornpun 2011; Vuong et al. 2006) or spectrophotometric methods using fruit extracts. Although these methods allow accurate quantification of individual carotenoids and separation of isomers; it is very laborious and this standardised methodology is not available to primary producers in developing nations where *M. cochinchinensis* is endemic. In contrast, colorimetry methods (Barba et al. 2006) could be used for rapid screening of carotenoid rich fruits since they are simple, cost effective and routinely used in the quantification of lycopene and β -carotene in other common fruits and vegetables (Biehler et al. 2010; Fish 2012) but not yet known for *M. cochinchinensis*.

The aims of this study were firstly, to elucidate the variation of lycopene and β -carotene concentration of *M. cochinchinensis* collected from Thailand, Vietnam and Australia. Secondly, to determine whether a colorimetry method could yield estimates of lycopene and β -carotene with comparable accuracy as methods accruing chemical extractions such as HPLC and spectrophotometry. The findings from this study will assist the breeding selection of cultivars with higher carotenoid levels and optimum growth environments to ensure consistent higher carotenoid contents. Furthermore, results from this study will also assist in the selection of carotenoid-rich fruits in resource-poor or technologically challenged situations.

Materials and methods

Sample collection

Mature fruits of 36 *M. cochinchinensis* samples belonging to 17 provinces of Thailand and Vietnam were collected (Table 1). All the fruits collected were at the same maturity stages of ripeness with orange or red coloured skin with soft texture and were collected by Dr Tien Huynh from their natural habitats, as reported by locals, during harvesting season (December–February). Eight fruits from a cultivated variety from Australia (A5-12) was provided by the Department of Primary Industries; NSW and used for comparison (Table 1). After collection, all the fruits were cleaned; the aril was separated from the seeds, transported to RMIT University in an insulated bag, separated into aliquots, covered with aluminium foil and stored at $-20\text{ }^{\circ}\text{C}$ in darkness until required. The geographical (altitude, latitude and longitude) and ecological (rainfall of wettest and driest month, observed minimum and maximum temperature, annual temperature range) data were obtained for each province using the DIVA-GIS spacial analysis software (Hijmans et al. 2004) (Table 1).

Extraction of carotenoids

Carotenoids of the *M. cochinchinensis* aril were extracted as previously described (Barba et al. 2006). The aril (2 g) was placed in a vessel protected from sunlight and mixed with 100 mL of extraction solvent (hexane/acetone/ethanol: 50:25:25 v/v/v) (Barba et al. 2006). The mixture was ultra-sonicated (Unisonics, Australia) for 30 min and then 15 mL of distilled water was added to enhance phase separation, where by the upper hexane layer contained the carotenoids and the bottom water layer contained hydrophilic compounds and cell debris. 1 mL of the upper organic layer was used for UV–visible spectroscopic analysis; 10 mL of the remaining hexane extract was

Table 1 Geographical and environmental climate of *M. cochinchinensis* from Vietnam (southern, northern and central), Thailand and Australia (Wimalasiri et al. 2016). Bioclimatic data was obtained from DIVA-GIS spacial analysis software (Hijmans et al. 2004)

Country	Province	Sample	Latitude	Longitude	Elevation (m)	Annual temperature °C			Precipitation (mm)		Climate ^a
						Max	Min	Difference	Annual	Wettest month	Driest month
S Vietnam	Can Tho	VS1	10.034320	105.775130	1	32.6	22.2	10.4	1524	254	2
	Can Tho	VS2	9.996850	105.665870	3	32.4	22.0	10.4	1623	269	3
	Vinh Long	VS3a, 3b	10.248758	105.969566	2	33.2	21.9	11.3	1413	244	2
	Tra On	VS4	9.965880	105.925140	3	32.5	22.1	10.4	1520	259	2
N Vietnam	HCM City	VS7	10.757410	106.673439	6	34.7	21.1	13.6	1851	310	2
	Hung Yen	VN8	20.814159	106.125992	4	33.1	14.4	19.0	1679	316	15
	Hung Yen	VN9a, 9b	21.648399	105.028999	71	32.2	11.6	20.6	1774	325	18
	Hai Duong	VN10	20.897711	106.423561	3	32.8	14.2	18.6	1648	311	17
	Hai Duong	VN11	20.919382	106.419062	3	33.0	13.5	19.5	1692	312	14
	PhuTho	VN12	21.331640	104.953453	296	30.8	10.5	20.3	1574	300	10
	PhuTho	VN13–15	21.445990	105.094419	296	32.3	11.3	20.9	1734	315	19
	Ha Noi	VN16–20	20.866699	105.750000	9	33.0	13.5	19.5	1700	314	14
	Ha Noi	VN21	20.985260	105.858501	9	33.1	13.5	19.6	1673	324	15
	Hoa Binh	VN22	20.829601	105.332893	195	31.6	11.7	19.9	1801	352	8
C Vietnam	Nam Dinh	VN24–27	20.422930	106.173630	4	33.4	14.7	18.7	1663	336	27
	Lam Dong	VC28, 29	11.835772	108.336275	1112	27.9	13.3	14.6	1651	248	7
	Lam Ha	VC32	11.793576	108.268610	925	28.1	13.8	14.3	1658	252	8
	Nakhon Pathom	T1, T3, T4	13.828790	100.039620	8	35.9	18.7	17.2	1237	257	6
Thailand	Samut Prakan	T5	13.576110	100.665420	2	34.5	20.4	14.1	1439	325	10
	Chiang Mai	T6, T8	18.767000	99.026161	316	36.2	12.9	23.3	1163	244	7
	Chanthaburi	T7	12.878721	102.129135	79	32.0	17.4	14.6	1751	316	10
	Australia ^b	Newcastle	33.212281	151.225069	NA	25.0	18.0	NA	NA	NA	NA

^a Regions with a minimum temperature <15 °C in coldest month was selected as temperate climate^b Plants grown in greenhouse condition with an average temperature of 25 °C

evaporated to dryness using a rotary evaporator (Büchi Labortechnik AG, Australia). The residue was dissolved in THF/acetonitrile/methanol: 50:25:25 v/v/v to a final volume of 4 mL for HPLC analysis. The final extract was filtered through a 0.45 µm PTFE membrane filter (Labquip, Australia) and a 20 µL injection was employed for HPLC analysis.

Analyses of carotenoids

High performance liquid chromatography (HPLC)

Carotenoid extracts were analysed via reverse phase analytical HPLC using solvents and conditions described previously (Barba et al. 2006). Samples were analysed using an isocratic method (90% CH₃CN/H₂O) on an Alltech Alltima HP C18 (250 × 4.6) 5 µm column at a flow rate of 1.0 mL/min. Analytical HPLC analyses were performed on a Dionex P680 solvent delivery system equipped with a PDA100 UV detector (operated using “Chromeleon” software). The column temperature was 30 °C and UV detection was monitored at 465 nm (provided highest peak areas for both lycopene and β-carotene). The lycopene and β-carotene contents was quantified with reference to samples of commercial standards of lycopene and β-carotene (Sigma Chemical, St. Louis, USA) of known concentrations ranging from 10 to 400 µg/mL which were used to obtain a calibration plot (Supplementary Fig. 2). The regression equation was used to determine the lycopene and β-carotene content of the samples where, $y = 1.16x - 4.369$, $R^2 = 0.971$ for lycopene and $y = 0.540x + 0.458$, $R^2 = 0.984$ for β-carotene. The analyses were performed in triplicate for all the samples and the results were presented as fresh weight basis (FW).

UV-visible spectrophotometry

Carotenoid extracts of *M. cochinchinensis* were placed in a 1 mL quartz cuvette and analysed using UV-visible spectrophotometry in a Cary® 50 UV-Vis spectrophotometer (Varian Inc, USA). UV visible spectra were recorded between 200 and 800 nm in hexane using a 1 nm interval.

The carotenoid content of the samples was calculated at the absorption maxima of lycopene (502 nm) and β-carotene (450 nm) using the equation: $C = (A_\lambda \times Fd)/\epsilon$ (Biehler et al. 2010), where C was the unknown content of carotenoids in mol/L; A_λ was the absorbance maximum of lycopene and β-carotene; F was a dilution factor adjusting for extraction, drying and reconstitution processes; ϵ was the molar extinction coefficient in hexane (ϵ (L/mol) = 169,000 for lycopene and 139,000 for β-carotene) (Zechmeister et al. 1943); and d was the width of the cuvette (1 cm). Using the molar mass of lycopene

(537 g/mol) and β-carotene (537 g/mol), the results were converted to mg/g fresh weight. The spectroscopic analyses were performed in triplicate for all *M. cochinchinensis* samples.

Colorimetry

The colour of the frozen aril samples was measured in triplicate using a tristimulus colorimeter (Konico Minolta, Chroma meter—CR 400, Japan) with a 8 mm measuring area and diffuse illumination/0° viewing angle. The data were recorded as L^* , a^* , b^* , corresponding to indices of lightness, redness and yellowness, respectively, according to the CIE $L^*a^*b^*$ scale (Hunter 1987). The L^* coordinate indicates darkness or lightness of colour and ranged from dark (0) to light (1). The a^* and b^* coordinates indicate the colour directions where $a^* > 1$ is the red direction and $a^* < 0$ is the green direction; $b^* > 1$ is the yellow direction and $b^* < 0$ is the blue direction (Itle and Kabelka 2009). Chroma indicates the saturation of colour, whereas as the value of chroma increases, the colour becomes more intense and as it decreases, the colour becomes duller. The hue was used to interpret the actual colour, for example, 0° = pure red, 45° = orange, 90° = yellow, 180° = pure green (Arias et al. 2000; Itle and Kabelka 2009). Both chroma and hue were derived from a^* and b^* values (Arias et al. 2000) where, $\text{chroma} = (a^{*2} + b^{*2})^{1/2}$ and $\text{hue} = \tan^{-1}(b^*/a^*)$. The contents of lycopene and β-carotene measured by HPLC and UV-visible spectrophotometry were compared graphically and statistically against colour values (L^* , a^* , b^* , chroma and hue) for the linear relationship using Minitab 17 software. For relationships that appeared to be non-linear, colour values were transformed (e.g. a^*/b^* , $(a^*/b^*)^2$, a^{*4}) to improve the linear relationships with the lycopene and β-carotene contents that was quantified by HPLC and UV-visible spectrophotometry.

Statistical analysis

All analytical experiments were performed in triplicate and the values for the contents of lycopene and β-carotene were analysed using the Minitab statistical software (version 17). For normally distributed data, the means were compared using the one-way analysis of variance (ANOVA). Non-normally distributed data were transformed (e.g. $\log_{10}$) or analysed using the non-parametric Kruskal–Wallis test and the significance difference between the means were analysed using Fisher’s post hoc test. Statistical values of $p \leq 0.05$ were considered as significantly different. 36 *M. cochinchinensis* samples grown in its natural habitat in Vietnam (southern, central and northern) and Thailand was used to correlate carotenoid contents with eco-geographical

factors. The Pearson's correlation was used to determine whether the carotenoids correlated with any of the eco-geographical factors (latitude, longitude, maximum temperature, minimum temperature, annual temperature range, annual precipitation, rainfall of wettest and driest month and elevation) or with the different analysis methods used (HPLC, UV–Vis spectrophotometry and colorimeter). The r value represented the correlation coefficient between two variables (1 = total positive correlation, 0 = no correlation, -1 = total negative correction) and the statistically significant level was set at $p \leq 0.05$. Principal component analysis (PCA) for lycopene and β -carotene content of 44 *M. cochinchinensis* samples was carried out using Minitab statistical software (version 17).

Results

Variation of carotenoid content in *M. cochinchinensis* based on collection sites

The carotenoid content of all tested samples was highly variable and ranged between 0.27 to 7.76 mg/g for lycopene and 0.20 to 8.27 mg/g for β -carotene (Fig. 1). The highest lycopene content was observed in samples

collected from the Ha Noi (7.76 mg/g) of Northern Vietnam and Lam Ha (6.45 mg/g) and Lam Dong (6.64 mg/g) provinces of Central Vietnam. These samples contained lycopene at contents more than 28 times higher than those from the Can Tho province (0.27 mg/g) in Southern Vietnam (Fig. 1). The highest β -carotene content was observed in a sample from Nam Dinh (9.60 mg/g) in Northern Vietnam which was 37 times higher than those from Chiang Mai province (0.20 mg/g) of Thailand (Fig. 1).

The proportions of lycopene and β -carotene were highly variable within the analysed fruits. Some fruits possessed high lycopene and low β -carotene (Lam Ha 6.46 mg/g lycopene and 1.77 mg/g β -carotene) contents whilst others had equally high (Hoa Binh 5.17 mg/g lycopene and 5.66 mg/g β -carotene) or equally low (Chanthaburi 0.88 mg/g lycopene and 0.82 mg/g β -carotene) contents. The fruits collected from most regions contained more lycopene than β -carotene (38 of 44 samples), with the highest difference being a 38-fold from the Chiang Mai region (Thailand).

The PCA analysis grouped the three Central Vietnam samples together (VC28, VC29 and VC32) and collectively contained the highest lycopene contents and was in agreement with the UPGMA cluster analysis previously reported (Wimalasiri et al. 2016). Samples from Southern

Fig. 1 Variation in lycopene and β -carotene content of 44 *M. cochinchinensis* samples collected from Australia (AU), Thailand (TH) and Vietnam (northern = VN, central = VC and southern = VS) detected by HPLC. Data are shown as mean + standard

deviation (SD) of three analytical replicates for an individual sample. * and # indicated statistical significance at $p \leq 0.05$ for lycopene and β -carotene, respectively compared with HPLC

Vietnam, Thailand and Australia was grouped together (Fig. 2) but the T1 and T3 samples were separated from the other Thailand samples due to the high β -carotene contents. The highest variation in lycopene and β -carotene content was observed in Northern Vietnam samples and was in agreement with genetic and morphological clusters (Wimalasiri et al. 2016). The Lam Dong province of Central Vietnam and the Hoa Binh province of Northern Vietnam possessed genotypes that contained high contents of lycopene and β -carotene, respectively. Therefore, these varieties should be considered for future commercial production. Up to date, no apparent genetic marker is identified for the superior lycopene and β -carotene contents in *M. cochinchinensis* from Central and Northern Vietnam. The contents of lycopene and β -carotene have been linked with the genes involved in their biosynthesis pathway in tomato (Smita et al. 2013), which has facilitated the identification of genotypes producing high carotenoid contents for commercial breeding programs (Smita et al. 2013). Future studies focused on the expression of key genes involved in lycopene (CRTISO PSY) and β -carotene (LCYB) accumulation will facilitate the selection of breeding stock of *M. cochinchinensis* for the development of consistently high carotenoid synthesising varieties.

The effect of environment factors on the variation of carotenoid content of *M. cochinchinensis*

The samples from Australia was excluded from analysis to determine the correlation between carotenoid content and eco-geographical factors since they were grown in controlled environments compared to the samples from Vietnam and Thailand which were grown in their natural

habitats. Of the seven environmental factors investigated in this study, temperature negatively influenced lycopene and β -carotene accumulation whilst elevation and precipitation positively influenced lycopene and β -carotene accumulation, respectively (Table 2; Supplementary Fig. 3).

Temperate cooler climates with low temperatures, high temperature fluctuations and high elevations were correlated with a high lycopene content (Table 2; Fig. 1). The highest lycopene content was observed in samples from Northern and Central Vietnam, which are cooler regions with low minimum temperatures ($<14^{\circ}\text{C}$) and high temperature ranges ($>18^{\circ}\text{C}$) (Table 1; Fig. 1). The correlation between minimum temperature and lycopene contents was moderate, with an r^2 value of 0.28, which can interpret that 28% of the variability of lycopene contents in *M. cochinchinensis* was due to the minimum temperature (Table 2). Samples from high elevations correlated with a high lycopene content, such as those from Lam Dong and Lam Ha, which are situated at 925–1112 m above sea level (Table 1; Fig. 1). In comparison, a low content of lycopene was seen in samples from Southern Vietnam, which has high minimum temperatures ($>20^{\circ}\text{C}$) reminiscent of tropical climates and consequently low annual temperature ranges ($<11^{\circ}\text{C}$) and low elevations (1–6 m).

The β -carotene content was significantly reduced in warmer tropical climates where the maximum temperatures exceeded 34°C ($p < 0.05$, $r = -0.416$) (Table 2) (e.g. Samut Prakan and HCM city). In contrast, a high β -carotene content was seen in samples from provinces that have cooler temperate climates (e.g. Lam Dong and Lam Ha). However, the β -carotene content was not correlated with elevation (Tables 1, 2). A high β -carotene content was also inversely correlated with moist conditions as measured by

Fig. 2 Score plot of lycopene and β -carotene of 44 *M. cochinchinensis* samples collected from Vietnam, Thailand and Australia constructed using the Principal Component Analysis method (PCA) with Minitab statistical software (Version 17). Country of collection was indicated as different colours; orange Southern Vietnam, blue Northern Vietnam, red Central Vietnam, green Thailand and olive green Australia

Table 2 Pearson correlation coefficients (r) and coefficient of determination (r^2) of carotenoids between eco-geographical factors (maximum and minimum temperature, annual temperature range, elevation and precipitation) and the carotenoid content of 36 *M. cochinchinensis* samples as measured by HPLC

Factor	Details	Correlation coefficient (r)		Coefficient of determination (r^2)	
		Lycopene	β -carotene	Lycopene	β -carotene
Temperature	Minimum	−0.53 ^a	−0.27 ^{NS}	0.28	0.07
	Maximum	0.32 ^{NS}	−0.42 ^a	0.10	0.17
	Range	0.40 ^a	0.28 ^{NS}	0.16	0.08
Elevation	Elevation	0.38 ^a	−0.06 ^{NS}	0.14	0.00
Precipitation	Annual	0.25 ^{NS}	0.40 ^a	0.06	0.16
	Driest month	0.29 ^{NS}	0.40 ^a	0.09	0.16
	Wettest month	0.21 ^{NS}	0.37 ^a	0.04	0.14

^a Significant at $p < 0.05$

^{NS} Not significant $p > 0.05$

the annual precipitation ($p < 0.05$, $r = -0.400$) and the precipitation during the wettest ($p < 0.05$, $r = -0.402$) and driest month ($p < 0.05$, $r = -0.371$).

Comparisons between quantification assays

The lycopene content measured by UV–visible spectrophotometry and colorimetry methods was not significantly different ($p < 0.05$) from colorimetry. L^* , a^* and b^* values showed significant relationships with lycopene measured by HPLC but the correlation was low. However, $(a^*/b^*)^2$ transformation of colour value resulted a more linear relationship (Fig. 3). The estimated content of lycopene by UV–visible spectrophotometry ($r = 0.802$, $p \leq 0.001$) and $(a^*/b^*)^2$ value of colorimetry, ($r = 0.734$, $p \leq 0.001$) was significantly correlated with HPLC (Table 3; Fig. 3).

With respect to β -carotene quantification, there was a low correlation between the spectrophotometry and chromatography ($r = 0.49$, $p \leq 0.001$) (Table 3) results. The untransformed a^* value and $(a^*)^4$ value showed significant relationship with β -carotene measured by HPLC but the correlation was low (Table 3) indicating that colorimetry measurements are not suitable for the estimation of β -carotene content in *M. cochinchinensis*.

Discussion

Variation of carotenoid content in *M. cochinchinensis* based on collection sites

The geographical distribution of the collected *M. cochinchinensis* fruit was related to the carotenoid content, with the best levels observed in samples from specific provinces; the diversity of carotenoid level was vast, ranging from 0.88 to 6.30 mg/g for lycopene and 0.25 to 5.70 mg/g for β -carotene. The carotenoid content was

significantly different between provinces with the highest lycopene contents found in the samples from Central Vietnam in Lam Ha province and the highest β -carotene contents measured in the samples from Northern Vietnam in Hoa Binh province. This distribution of carotenoid content was related to that of the morphology from the same provinces, with a notable character being that Central Vietnam samples had the heaviest and largest seeds (Wimalasiri et al. 2016). The same samples were genetically and morphologically clustered (Wimalasiri et al. 2016), and was reflected in PCA analysis with high lycopene and β -carotene contents. The highest diversity in lycopene and β -carotene contents was seen in the samples from Northern Vietnam and was in agreement with genetic and morphology clustering (Wimalasiri et al. 2016). However, this diversity might be attributed by the larger number of Northern Vietnamese samples analysed in this study. Interestingly, the samples collected from Australia had similar contents of lycopene and β -carotene as did the Southern Vietnam samples and was grouped together indicating that the variety might have an influence on the nutrition content. However, the Thailand samples were not separated from the samples from Australia and Southern Vietnam as did for the genetic and morphology clustering indicating that there might be other factors, such as soil nutrition, micro-climatic differences and limited sampling per each location that are responsible for variations in carotenoids.

All samples analysed were genetically diverse based on molecular markers (Wimalasiri et al. 2016). However, there is no apparent genetic marker identified for lycopene content in *M. cochinchinensis*, which could make the plant produce fruits with consistent higher yields of lycopene as observed for samples from Lam Ha province compared with Chanthaburi province. Studies on other carotenoid rich foods, such as sweet potato, pumpkin squash and tomato, have also found that the genotype and variety determined the lycopene, phenolic compounds and

Fig. 3 Scatter plots showing correlations between carotenoids (lycopene and β -carotene) of 44 *M. cochinchinensis* aril extracts and analytical methods. **a** Scatter plot showing correlations between lycopene and UV-Vis spectrophotometry. **b** Scatter plot showing

correlations between lycopene and $(a^*/b^*)^2$. **c** Scatter plot showing correlations between β -carotene and UV-Vis spectrophotometry. **d** Scatter plot showing correlations between β -carotene and $(a^*)^4 \times 10^6$

Table 3 Pearson correlation coefficients (r) between colorimetry, spectrophotometry and chromatography (HPLC) of 44 *M. cochinchinensis* samples

Analytical test	Details	Correlation (r) with carotenoids (HPLC)		Correlation of determination (r^2) with carotenoids (HPLC)	
		Lycopene	β -carotene	Lycopene	β -carotene
Colorimetry	L*	−0.512 ^a	−0.031 ^{NS}	0.26	–
	a*	0.311 ^a	0.408 ^a	0.10	0.17
	b*	−0.412 ^a	0.127 ^{NS}	0.17	0.02
	a^*/b^*	0.686 ^a	0.069 ^{NS}	0.46	–
	$(a^*/b^*)^2$	0.734 ^a	0.063 ^{NS}	0.54	–
	Hue	−0.161 ^{NS}	−0.017 ^{NS}	–	–
	Chroma	−0.216 ^{NS}	0.188 ^{NS}	–	–
	$(a^*)^2$	0.302 ^a	0.418 ^a	0.09	0.17
	$(a^*)^4$	0.288 ^{NS}	0.427 ^a	–	0.18
	$(a^*)^{0.5}$	0.315 ^a	0.402 ^a	0.10	0.16
Spectrophotometry	a^*b^*	−0.216 ^{NS}	0.283 ^{NS}	–	–
	502 nm	0.802	NA	0.64	–
	450 nm	NA	0.490	–	0.24

^a Significant at $p < 0.05$

^{NS} Not significant at $p > 0.05$

NA Not available

antioxidant superiority of these fruits (Chandra et al. 2012; Lenucci et al. 2006; Martínez-Valverde et al. 2002; Rodríguez-Amaya and Kimura 2004). However, this is contradictory to other studies, which have suggested that the same varieties grown in separate countries with different climates had a diverse carotenoid content, indicating that geographical location had a greater impact than the variety (Nishiyama et al. 2005; Raffo et al. 2006; Rodríguez-Amaya and Kimura 2004; Sass-Kiss et al. 2005). This information on varietal differences and downstream influences on carotenoid accumulation was previously unknown for *M. cochinchinensis*. Further experiments are pivotal to elucidate if genetically identical varieties could be encouraged to produce higher lycopene contents for this potentially valuable crop.

The carotenoid contents of *M. cochinchinensis* samples analysed in this study were higher than expected and were 3 times higher for lycopene and 8 times higher for β -carotene compared to that previously reported for *M. cochinchinensis* (Aoki et al. 2002; Ishida et al. 2004; Kubola and Siriamornpun 2011). The global carotenoid market value was estimated to be \$1.5 billion in 2014 and it is expected to reach nearly \$1.8 billion in 2019, with an annual growth rate of 3.9% (BCC 2015). *M. cochinchinensis* can contribute to this expanding carotenoid market as an alternate source of lycopene and β -carotene. This information will be imperative for primary producers to facilitate the conservation, sharing and breeding of better varieties of *M. cochinchinensis* with consistently higher carotenoids.

The effect of environmental conditions on variation of carotenoid content in *M. cochinchinensis*

Of the seven environmental factors analysed, the temperature, elevation and precipitation were related to the carotenoid content of the *M. cochinchinensis* aril and therefore, they may have influenced the specific metabolic pathways for the production of lycopene, β -carotene or both. The optimum temperatures for lycopene synthesis are known to be between 16 and 21 °C for tomatoes (Martínez-Valverde et al. 2002) and, at temperatures above 30 °C, lycopene is known to be converted to β -carotene (Brandt et al. 2006; Dumas et al. 2003; Nishiyama et al. 2005). Therefore, high temperatures may have caused the low lycopene content observed in the samples from tropical Southern Vietnam and Thailand provinces, where the maximum temperatures exceed 30 °C compared to the samples from the temperate regions of Central Vietnam where the temperatures does not exceed 28 °C. Low temperatures at high altitudes with high UV radiation, particularly in Central Vietnam where the varieties are grown in mountainous provinces, may explain the high contents of

carotenoids. This was consistent with other studies, which indicated that low temperatures, high altitudes and high UV radiation increased secondary metabolite synthesis in other fruits such as tomato, grape berry and passionfruit (Jaakola and Hohtola 2010; Rodríguez-Amaya and Kimura 2004). The influence of geography and climate has been found to override genetic control in tomatoes (Aherne et al. 2009) and this finding has been implemented using seasonal and environmental manipulations to improve lycopene synthesis and bio-accumulation (Raffo et al. 2006).

Interestingly, a *M. cochinchinensis* variety originated from Southern Vietnam (Wimalasiri et al. 2016) but grown in temperature-controlled glasshouses at 25 °C in Australia did not produce higher contents of lycopene than the samples collected from Southern Vietnam. This suggests that variables other than temperature may be involved in carotenoid synthesis and bioaccumulation in *M. cochinchinensis*. Since there is a lack of genetic markers to predict higher carotenoid content, eco-geographical contributions could assist primary producers to maximise the carotenoid production. More experiments are currently underway with the same genotypes growing in different provinces to determine the dominance of eco-geographic versus genetic influences on lycopene accumulation.

Comparisons between quantification assays

Selection of fruits and cultivars with higher carotenoid content require rapid analysis methods which are suitable to use in field conditions. The estimation of lycopene in the aril of *M. cochinchinensis* was consistent across the chromatographic, colorimetric and spectrophotometric methods. High performance liquid chromatography (HPLC) is the standard method to quantify carotenoids in fruits and vegetables but it is too costly and inefficient for resource poor farmers. The use of colorimetry and spectrophotometry to estimate lycopene is more feasible and this has been implemented in field applications for tomatoes (Arias et al. 2000) and cucurbits such as pumpkins and squash (Itle and Kabelka 2009). These methods have been used to detect differences among genotypes (Hyman et al. 2004). Therefore, colorimetry method can be used to select lycopene rich *M. cochinchinensis* fruits in farms and genotypes with higher lycopene as breeding stock.

In comparison, the estimation of β -carotene was consistent with spectrophotometry but not colorimetry, compared with HPLC. The inconsistency of the colorimetry results may be due to interference from a high lycopene content. This has been confirmed in other carotenoid studies when lycopene, xanthophyll and chlorophyll were co-localised in homogenates (Arias et al. 2000; Fish 2012; Hyman et al. 2004). Therefore, the colorimetry method may require additional separation, fractionation and purification

for the accurate quantification of β -carotene in *M. cochinchinensis*.

Conclusion

This study confirms the nutritional variation of *M. cochinchinensis* from wild and cultivated samples obtained from Vietnam and Thailand. The fruit contained up to 200 times more lycopene than hydroponic tomatoes and 54 times more β -carotene than carrots than previously reported. The carotenoid contents in this study were more than expected; they were 3 times higher in lycopene and 8 times higher in β -carotene than the highest content previously reported for *M. cochinchinensis*. Varieties with high carotenoids (lycopene and β -carotene) and grown under optimal eco-geographical parameters (optimum temperature, elevation and precipitation) were identified. These findings will be pivotal for the agricultural development of this fruit for the food and nutraceutical industries.

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