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Abstract

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Article 103721

Journal of Food Composition and Analysis 96 (2021) 103722

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Original Research Article

Carotenoid composition in buah merah (*Pandanus conoideus* Lam.), an indigenous red fruit of the Papua Islands

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ARTICLE INFO

Keywords:
Red fruit
Pandanus conoideus
κ-end group carotenoids
Carotenoid analysis
Biosynthesis
Chromatographic and spectrometric analyses

ABSTRACT

The composition of carotenoid pigments from the fruit of the *Pandanus* plant, *Pandanus conoideus* Lam. monocot or locally named as red fruit, indigenous to the Papua Islands, was investigated. By chromatographic and spectrometric analyses using RP-HPLC with C18 and C30 columns and gradient elution, NMR, MS/MS and FT-IR, eight κ-end group carotenoids, 5,6-diepicapsokarpoxanthin, capsorubin, capsanthin, cryptocapsin, 13-*cis* capsorubin, and three capsanthin epoxides were identified. Additionally, β-cryptoxanthin 5,6-epoxide, α- and β-cryptoxanthin, α- and β-carotene, and other carotenoid-type compounds were found. The κ-end group carotenoids in red fruit comprised 92 % in relative contents and was much higher than that in red chili pepper, *Capsicum annuum* L., at 64 %. These findings indicate that red fruit has similar carotenoids to those of red chili pepper, not only in composition but also in the content. Xanthophyll cycle carotenoids antheraxanthin and violaxanthin are the precursors of capsanthin and capsorubin, respectively. However, these precursors, as well as the precursors of zeaxanthin, were not detected in the chromatographic separation and identification in the extracts of red fruit, although they were detected in red chili pepper under the same analytical conditions used. Therefore, in red fruit, those precursors are likely not responsible for the biosynthesis of κ-end group carotenoids.

1. Introduction

Pandanus conoideus Lam. monocot is an indigenous *Pandanus* plant of the Papua Islands and widely spreads in Maluku, Papua Indonesia and Papua New Guinea (Murtiningrum et al., 2012). The fruit of *P. conoideus*, or locally named buah merah, called hereafter red fruit, is formed by a deep red color of the integrated compound, called syncarp (86–110 cm in length and 30–35 cm in girth), with a right-triangular cylindrical shape and comprising many single fruits, called drupe (1.0–1.5 cm in length) (Sianipar and Santosa, 2016) (Fig. 1). The people of the Papua Islands utilize the red fruit as a colorant to give a strong red color to food and food products, functional food and edible oil sources, as well as traditional medicines, from its oil (Hyndman, 1984). Moreover, red fruit oil has been reported for its potential health benefits in animal models, i.e., preventing the symptoms of preeclampsia (Sugiritama et al., 2016) and increasing the quality of spermatozoa (Widayati et al., 2018). These biological activities may correlate to the active compounds present in the red fruit, i.e., fatty acids, tocopherols, and carotenoids (Sarungallo et al., 2015a, b).

The rapid improvements in analytical instruments have enabled the structure elucidation of carotenoids in nature (Maoka and Akimoto, 2008). Annually, more than ten new carotenoids have been elucidated. Approximately 700 naturally occurring carotenoids have been reported in 2004 (Britton et al., 2004), and the number of natural carotenoid structures has increased to 850 in 2018 (Maoka, 2020). The structural diversity of carotenoids was also reported in non-photosynthetic organs,

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https://doi.org/10.1016/j.jfca.2020.103722
Received 24 April 2020; Received in revised form 4 October 2020; Accepted 12 November 2020
Available online 19 November 2020

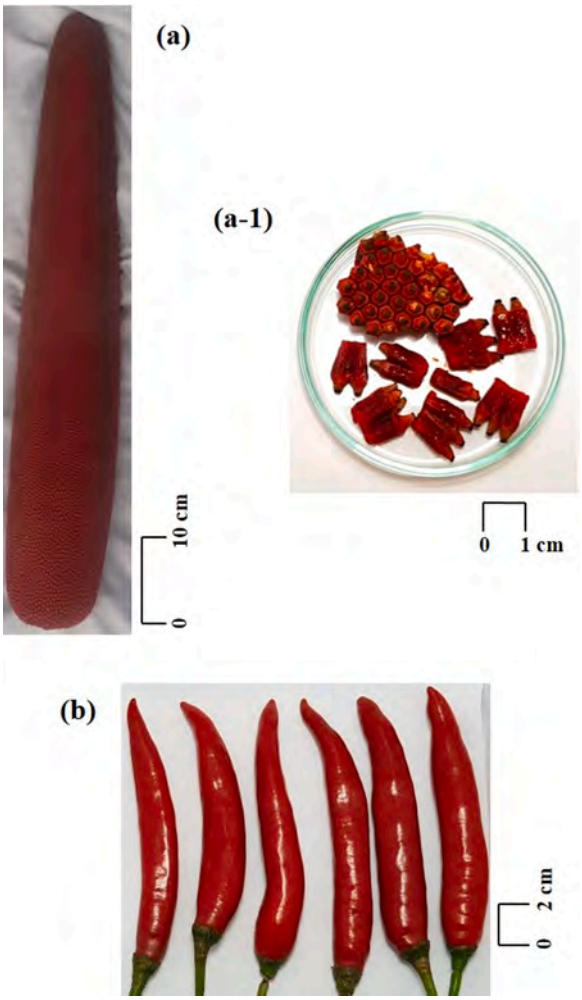

Fig. 1. Photos of harvested red fruit (*P. conoideus*) and red chili pepper (*Capsicum annuum*). (a) a cylindrical syncarp from the harvested red fruit; (a-1) a single fruit (drupe) and its bunches from red fruit; and (b) fruits of red chili pepper.

such as fruits, seeds, and flowers (Maoka, 2009).

The carotenoids from the fruits of red paprika and chili pepper have attracted attention due to their deep and bright red-colored fruits (Arimboor et al., 2015; del Gómez-García and Ochoa-Alejo, 2013; Hassan et al., 2019). They comprise capsanthin, capsorubin, and cryptocapsin, carotenoids that contain at least one κ-end group (κ-cyclic carotenoids) as the major component (Davies et al., 1970; Deli et al., 2001). These carotenoid compositions are typical for *Capsicum* species (Deli and Molnár, 2002). In addition to *Capsicum* species, several plants have been determined to partly have a biosynthetic pathway of κ-cyclic carotenoids. Capsanthin and capsorubin were found in the fruits of *Asparagus officinalis* (Deli et al., 2000) and tepals of tiger lily (*Lolium lancifolium*) (Jeknić et al., 2012). Moreover, capsanthin was found in the fruit of *Berberis* sp. (Bubicz, 1965). Cryptocapsin is the main carotenoid component together with a typical carotenoid sapotexanthin from mamey fruit (*Pouteria sapota*) (Chacón-Ordóñez et al., 2017; Murillo et al., 2013). Cryptocapsin and β-cryptoxanthin were also found in maracuya chino fruit (*Cionosicyos macranthus*) at high amounts (Murillo et al., 2013).

In common *Pandanus* species (*Pandanus tectorius*), the high concentrations of α- and β-carotenes, as well as β-cryptoxanthin, have been quantified in edible flesh cultivars with orange-red, orange and yellow color (Englberger et al., 2003, 2006). Until now, in red fruit, another *Pandanus* plant indigenous in the Papua Islands, only minor carotenoids, α- and β-cryptoxanthins and α- and β-carotenes as pro-vitamin A carotenoids, have been reported (Sarungallo et al., 2015b). A dark-red color of red fruit may indicate the presence of the red carotenoids, such as κ-cyclic carotenoids. However, no information is available concerning the major carotenoid species from red fruit. In the present study, we separated and identified the main carotenoids from red fruit and found the accumulation of several carotenoids containing a κ-ring as end groups and their intermediates in the biosynthetic pathway. Compositional similarity of the carotenoids in the red fruit to those of fruit of red chili pepper is also discussed.

2. Materials and methods

2.1. Materials

Red fruit with a deep red color of cylindrical syncarp was collected at Post 7, Sentani District, Jayapura Regency, Papua Province, Indonesia on June 10, 2016 (Fig. 1). The collected red fruits were immediately brought back to the Laboratory of MRCPP, and then the fruits were stored at −20 °C in the dark. The red fruit was identified as *Pandanus conoideus* using the *matK* sequence and phylogenetic analysis. The sequence was deposited in the NCBI GenBank database under the accession number MN692945.1. Deep red-colored ripe fruits of red chili pepper were purchased from the local market, Malang, East Java, Indonesia.

The solvents methanol (MeOH) and methyl tertiary butyl ether (MTBE), used for liquid chromatography-mass spectrometry (LC-MS) were of LC grade. Other solvents and reagents used were of analytical grades and were obtained from Merck (Darmstadt, Germany). KBr crystal of spectroscopy grade was obtained from JASCO (Tokyo, Japan), and N_2 gas of ultrahigh purity grade was from Samator (Sidoarjo, Indonesia).

The standard pigments, violaxanthin, antheraxanthin, zeaxanthin, lutein, α-carotene, and β-carotene, were obtained from NATChrom (Malang, East Java, Indonesia). β-Cryptoxanthin was purified from the peels of mandarin orange.

2.2. Carotenoid extraction and saponification

Approximately 0.2 *g* of the exocarp of drupe was extracted with a mixture containing 1 mL of ethanol (EtOH) and *n*-hexane (1:1, v/v) containing a few grains of sodium L-ascorbate and calcium carbonate in a 2-mL centrifuge tube. The solution containing the exocarp of drupe was homogenized using a polytetrafluoroethylene pestle, and then extraction was carried out using a vortex for 2 min at room temperature. The carotenoid extract was separated from the residue by centrifugation (6000 *g* for 1 min at room temperature). The supernatant containing carotenoids was collected, and then extraction of the residue was continued with the addition of 1 mL of extraction solvent as described above. The extraction was repeated for 3–4 times until the residue became colorless. The combined carotenoid extracts were dried using N_2 gas. Saponification of the extract was carried out according to the method of Kurniawan et al. (2019). The saponified carotenoids were dried under a stream of N_2 gas, and the dried carotenoid extracts were kept at −20 °C in the dark for further high-performance liquid chromatography (HPLC) analysis.

For the red chili pepper carotenoids, red chili pepper was ground using a blender into small pieces and then approximately 0.2 g of sample was used for carotenoid extraction. The extraction procedure was the same as that for the red fruit until the first extraction with the ethanol and *n*-hexane mixture. The upper organic phase containing carotenoids was collected, and the extraction was then repeated at least 3–4 times with the addition of 0.5 mL of *n*-hexane until the residue became colorless. The combined carotenoid fractions were dried using N_2 gas. The saponification procedure was the same as that for the red fruit.

2.3. Carotenoid separation

The separation of carotenoids from unsaponified and saponified sample extracts was carried out by the modified method of Deli et al. (2001) using an analytical reversed-phase (RP)-HPLC (LC-20AD) system equipped with a photodiode array detector (Shimadzu, Kyoto, Japan) through a Cosmosil 5C18-MS-II column (150 × 4.6 mm I.D.) (Nacalai Tesque, Kyoto, Japan) with a gradient elution program using a mixture of H_2O, MeOH and acetone at a column oven temperature of 30 °C and a flow rate of 1 mL/min. The gradient elution program used was as follows: 12 % H_2O/88 % MeOH for 8 min, to 9.6 % H_2O/90.4 % MeOH for 8 min, to 6% H_2O/94 % MeOH for 8 min, to 100 % MeOH for 7 min, 100 % MeOH for 2 min, to 50 % MeOH/50 % acetone for 6 min, and 50 % MeOH/50 % acetone for 11 min. The carotenoid extracts dissolved in MeOH was injected into the RP-HPLC column.

According to the general procedures, we used EtOH and *n*-hexane (polar and non-polar) mixture to extract effectively a wide range of carotenoid species and selected MeOH (polar) for injection solvent to gain high resolution of the separation in RP-HPLC. In this case, good separation is achieved, but quantitatively low due to the used polar injection solvent that is not able to quantitatively dissolve the nonpolar carotenoids. Thus, the differences in the solvent strength between the extraction and injection were dilemma faced between quantitation and separation efficiency and the suboptimal work-up procedures used here may lead to potential limitation. To overcome this, we carried out two HPLC runs for the same sample under different conditions.

To confirm the results obtained using the Cosmosil C18 column, the analysis was carried out using a YMC Carotenoid column (150 × 4.6 mm I.D.; 3 μm particle size) (YMC, Kyoto, Japan). This method is specialized for the separation of xanthophyll cycle carotenoids and enables clear separation, particularly for three carotenoids, violaxanthin (t_R =7.14 min), antheraxanthin (t_R =11.27 min) and zeaxanthin (t_R =17.44 min). The analysis was also performed using a high concentration of the sample to avoid detection limits as mentioned above. The equipment used was the same as that described above. A mixture of H_2O, MeOH and MTBE was used as the mobile phase and was eluted with the following gradient program by volume: 4:90:6 at 0 min, 4:85:11 at 10 min, 4:50:46 at 20 min, 4:6:90 at 70 min, at a column temperature of 30 °C and a flow rate of 1 mL/min.

The peak number is expressed for both samples of red fruit and red chili pepper according to the total number of detected peaks in the order of increasing retention time (t_R) to examine the similarity and simplify the identification. The t_R of both samples was adjusted using an internal standard, *β*-carotene, which is known to be present in both samples (Deli et al., 2001; Sarungallo et al., 2015b). The identification of carotenoids was based on its t_R and absorption properties, spectral shape and maximal absorption wavelengths (λ_{max}) compared with the HPLC results of carotenoids from red chili pepper and the reference (Deli et al., 2001). The experiments were performed at least three times independently, and statistical treatments were carried out, particularly for the relative contents and t_R.

2.4. Purification and analysis of main carotenoids

The main carotenoids from red fruit were purified by RP-HPLC (LC-20AD) equipped with a photodiode array detector (Shimadzu) and a YMC Carotenoid column (150 × 4.6 mm I.D.; 3 μm particle size) connected to a guard column. A gradient elution program of a mixture of H_2O, MeOH and MTBE was applied as follows: 4:81:15 (v/v/v) at 0 min to 4:6:90 (v/v/v) at 70 min at a column oven temperature of 30 °C and a flow rate of 1 mL/min. The main carotenoids were eluted as single peaks with a t_R of 3.83 min (carotenoid #1), 5.68 min (carotenoid #2), 7.48 min (carotenoid #3), 8.62 min (carotenoid #4), and 14.83 min (carotenoid #5), and peaks were tentatively designated as those in parentheses. Each peak was collected and highly purified to more than 96 %, as estimated by the peak areas of the HPLC chromatograms. Finally, the peaks were separated and identified by analytical HPLC using a Cosmosil 5C18-MS-II column (Nacalai Tesque) as described above. The purified carotenoids well corresponded to peaks of the analytical HPLC (cf. Table 1), except for carotenoid #2, as follows: carotenoid #1 = peak 17; carotenoid #3 = peak 21; carotenoid #4 = peak 31; and carotenoid #5 = peak 52. Carotenoid #2 is a mixture of three components, and the purified main peak corresponded to peak 29. Hereafter, the peak numbers obtained by analytical RP-HPLC using a Cosmosil 5C18-MS-II column were used. Similarly, the capsanthin epoxide group, peaks 23, 27, and 28, and *β*-cryptoxanthin 5,6-epoxide, peak 54, were purified to approximately 96 % by analytical RP-HPLC as described above in the carotenoid separation.

The concentrations of two major carotenoids, capsorubin and capsanthin, were determined by linear equations using capsorubin (y = 162.24x - 97.549, R^2 = 0.9987) and capsanthin (y = 209.33x + 45.327, R^2 = 0.9983), where x is the pigment concentration (μg/mL) and y is the peak area ($\times 10^{-3}$) at their λ_{max} values of 483 and 472 nm, respectively. The determinations were carried out at least three times, and the means ± SE are shown on a g dry-weight basis.

2.5. Carotenoid characterization

The main carotenoids of red fruit were characterized using the following instruments, i.e., ultraviolet-visible (UV-vis) absorption, Fourier-transform infrared (FT-IR) and circular dichroism (CD) spectrophotometers, electrospray ionization-liquid chromatography triple quadrupole mass spectrometry LC-ESI-MS/MS, matrix-assisted laser desorption/ionization (MALDI)-MS, electrospray ionization-time-of-flight (ESI-TOF)-MS, and ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR). The measurements were conducted at room temperature under dark conditions. Subsequently, the data obtained were analyzed using Plots32 Version 1.35 software.

2.5.1. UV-vis spectroscopy

The absorption spectrum of the isolated carotenoid was measured using a UV-vis spectrophotometer UV-1700 (Shimadzu) in the wavelength interval of 200–900 nm, a medium scan speed, 1 nm of data points and a cell-path length of 10 mm. The dried carotenoid was measured after dissolving separately with EtOH, acetone, and *n*-hexane.

2.5.2. FT-IR spectroscopy

An FT-IR spectrophotometer 6800 type A (JASCO) equipped with a TGS detector was used for transmittance measurement in the 4000 to 400 cm^{-1} wavenumber regions. The FT-IR measurement was carried out using KBr pellets at 4 cm^{-1} resolution with 128 scans. The finely pulverized KBr crystal was mixed with the dried carotenoid, and then the mixture was placed into a pellet-forming die.

2.5.3. CD spectroscopy

CD spectra were recorded at 20 °C using a J-720W spectropolarimeter (JASCO) with a 1.0-nm excitation bandwidth according to the method of Yamano et al. (2018). The dried carotenoid was dissolved in EtOH for CD measurement.

2.5.4. ESI-MS/MS analysis

The highly purified compounds (> 96 % purity) were analyzed by ESI-MS/MS using a triple quadrupole mass spectrometer LCMS-8030 (Shimadzu). The separation of carotenoid was carried out using a Cosmosil 2.5Cholester column (50 × 2.0 mm I.D.) (Nacalai Tesque) with an isocratic elution program using 0.1 % formic acid in H_2O (10 %) and 0.1 % formic acid in MeOH (90 %) at a column oven temperature of 30 °C and a flow rate of 0.4 mL/min. For the analysis of nonpolar carotenoids from *β*-cryptoxanthin 5,6-epoxide to *β*-carotene, a different method was employed using a YMC C30 column (100 × 4.6 mm I.D.; S-3 μm) with a mobile phase, MeOH containing 0.1 % formic acid and MTBE and gradient elution (15 % MTBE at 0 min to 58 % MTBE at 40 min) at a

Table 1
Summary of carotenoid separation and identification in red fruit and red chili pepper by HPLC. The peak numbers correspond to those of the elution profiles in Fig. 2. Experiments were carried out more than 3 times independently, and their averages are shown for t_R and relative peak area (%). The peak area was detected at 480 nm.

Peak No	t_R* (min)	Red Fruit		Red Chili Pepper		Precursor ion (m/z)	Fragment ion (m/z)	Carotenoid
		λ_{max} (nm)	Relative peak area ** (%)	λ_{max} (nm)	Relative peak area *** (%)			
17	6.11	-, 470, -	1.68	-, 468, -	0.25	619.55 $[M+H]^+$	601.60 $[M+H–H_2O]^+$; 583.65 $[M+H–2H_2O]^+$	5,6-diepicapsokarpoxanthin (Carotenoid #1)
21	7.70	-, 483, -	71.17	-, 482, -	2.99	601.60 $[M+H]^+$	583.70 $[M+H–H_2O]^+$	Capsorubin (Carotenoid #3)
23	8.36	-, 470, -	0.15	-, 469, -	0.93	601.50 $[M+H]^+$	583.50 $[M+H–H_2O]^+$	Capsanthin 5,6-epoxide
24	8.74	–	–	-, 445, 472	2.08	602.65 $[M]^+$	584.80 $[M–H_2O]^+$	5,6-diepikarpoxanthin
26	8.96	–	–	417, 439, 468	1.01	601.60 $[M+H]^+$	583.55 $[M+H–H_2O]^+$	Violaxanthin
27	9.04	-, 453, -	0.08	-, 454, -	0.14	601.60 $[M+H]^+$	583.40 $[M+H–H_2O]^+$	Capsanthin 5,8-epoxide
28	9.50	-, 471, -	2.29	-, 470, -	2.76	601.55 $[M+H]^+$	583.40 $[M+H–H_2O]^+$	Capsanthin 3,6-epoxide
29	9.59	374, -, 476, -	3.74	–	–	601.65 $[M+H]^+$	583.70 $[M+H–H_2O]^+$	13-*cis* isomer of capsorubin (Carotenoid #2)
31	10.09	-, 474, -	9.65	-, 475, -	56.65	585.45 $[M+H]^+$	567.70 $[M+H–H_2O]^+$	Capsanthin (Carotenoid #4)
36	10.78	–	–	419, 440, 469	0.13	585.45 $[M+H]^+$	567.50 $[M+H–H_2O]^+$	Antheraxanthin
38	11.12	–	–	-, 427, 453	0.35	584.60 $[M]^+$	492.75 $[M–92]^+$	(*8R*) mutatoxanthin
39	11.48	–	–	-, 427, 451	0.16	584.60 $[M]^+$	492.75 $[M–92]^+$	(*8S*) mutatoxanthin
40	11.79	–	–	-, 445, 472	6.05	568.60 $[M]^+$	551.50 $[M+H–H_2O]^+$; 476.45 $[M–92]^+$	Lutein
42	12.07	–	–	-, 450, 477	15.63	568.75 $[M]^+$	476.35 $[M–92]^+$	Zeaxanthin
52	16.28	-, 472, -	2.92	-, 473, -	0.09	569.50 $[M+H]^+$	551.75 $[M+H–H_2O]^+$	Cryptocapsin (Carotenoid #5)
54	17.49	-, 444, 471	0.01	-, 445, 471	0.02	568.30 $[M]^+$	560.25 $[M–H_2O]^+$	*β*-cryptoxanthin 5,6-epoxide
55	18.02	-, 443, 473	0.02	-, 444, 473	0.14	552.80 $[M]^+$	–	*α*-cryptoxanthin
56	18.41	-, 449, 475	0.07	-, 450, 477	2.82	552.60 $[M]^+$	460.40 $[M–92]^+$	*β*-cryptoxanthin
59	27.58	-, 445, 473	0.01	-, 445, 473	0.09	536.65 $[M]^+$	444.35 $[M–92]^+$	*α*-carotene
60	28.23	-, 450, 476	0.14	-, 450, 476	2.65	536.60 $[M]^+$	444.50 $[M–92]^+$	*β*-carotene

–, Not detected.
* CV < 0.51 %.
** CV < 22.1 %.
*** CV < 3.49 % for the assigned *κ*-cyclic carotenoids.

column oven temperature of 30 °C and a flow rate of 0.5 mL/min.

Mass analysis was performed in the ESI mode in the mass range from 50 to 700 m/z. Other parameters for mass analysis were used as described previously (Brotosudarmo et al., 2018). The identification of the carotenoids by MS analysis was based on the precursor and fragment ions using a mode of Q1 and Q3 scans (+), a product ion scan with −10 eV of collision energy (CE), and single ion monitoring (SIM) at the m/z of precursor ions. The isolated carotenoid was identified by comparing the recorded chromatographic and spectral data with the data on the carotenoid standard stored in the library using LabSolution LCMS ver. 5.4 (Shimadzu). The software compared t_R and aligned MS/MS data to calculate a match factor and produce a degree of similarity between spectra.

2.5.5. *MALDI-MS and TOF-MS analysis*

For peaks 21, 31, and 52, high-resolution MALDI-MS spectra were acquired using a 7 T SolariX FTICRMS system (Bruker Daltonik GmbH, Bremen, Germany), with an estimated resolving power of 94,000 at m/z 400 using DCTB (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) as a matrix. The ion formulae were determined using the smart formula algorithm in Data Analysis version 4.4 software (Bruker). For peak 17, ESI-TOF-MS spectra were acquired by scanning from m/z 100 to 1500 with a capillary voltage of 3.2 kV, a cone voltage of 40 eV, and a source temperature of 120 °C.

2.5.6. *^{1}H- and ^{13}C-NMR analysis*

The ^{1}H- and ^{13}C-NMR spectra were measured using a Varian Unity Inova 500 spectrometer (Varian Corporation, Palo Alto, CA, USA) for peaks 17 and 31 in deuterated chloroform ($CDCl_3$) with tetramethylsilane (TMS) as an internal standard according to Koizumi et al. (2018). Peaks 21 and 52 were measured using an AVANCE III 600 MHz cryo-probe system (Bruker BioSpin, Rheinstetten, Germany).

2.6. *13-cis isomer of capsorubin formation assay*

The 13-*cis* isomer of capsorubin was prepared by thermal isomerization of the purified capsorubin. Pure capsorubin (≥ 98 %) was dissolved in EtOH and incubated at 90 °C for 120 min. Next, 13-*cis* isomer was purified by HPLC using a YMC Carotenoid column under the same conditions described in carotenoid purification.

2.7. The spectroscopic data for structural identification

5,6-diepicapsokarpoxanthin (peak 17)

((*3S,5S,6S*,3′S,5′R)-*3,5,6,3′-Tetrahydroxy-5,6-dihydro-β,κ-caroten-6-one*). Available amount: ca. 0.05 mg. UV/VIS (acetone): 494, 467. ESI-TOF-MS, *m/z* (rel. int.): 641.4182 ($C_{40}H_{58}O_5Na$, Calc. 641.4182, 14, $[M + Na]^+$), 619.4362 ($C_{40}H_{59}O_5$, Calc. 619.4362, 17, $[M+H]^+$), 601.4249 (100, $[M+H-H_2O]^+$), 583.4139 (72, $[M+H-2H_2O]^+$), 565.4028 (14, $[M+H-3H_2O]^+$), 527.3521 (4, $[M+H-92]^+$). ESI-MS/MS, *m/z* (rel. int.): 673.85 ($C_{41}H_{62}O_6Na$, Calc. 673.4444, 23, $[M + Na + CH_3OH]^+$), 641.75 ($C_{40}H_{58}O_5Na$, Calc. 641.4182, 61, $[M + Na]^+$), 619.55 ($C_{40}H_{59}O_5$, Calc. 619.4363, 58, $[M+H]^+$), 601.60 ($C_{40}H_{57}O_4$, Calc. 601.4257, 100, $[M+H-H_2O]^+$), 583.65 ($C_{40}H_{55}O_3$, Calc. 583.4151, 40, $[M+H-2H_2O]^+$). ^{1}H-NMR δ(500 MHz, $CDCl_3$, including ^{1}H^{1}HCOSY): 0.84 (3H, s, H-16′), 0.90 (3H, s, H-16), 1.13 (3H, s, H-18), 1.21 (3H, s, H-17′), 1.32 (3H, s, H-17), 1.37 (3H, s, H-18′), 1.49 (1H, *dd*, *J* = 15, 4 Hz, H-4′ax), ca. 1.65 (1H, not determined due to signal overlap, H-2ax), 1.71 (1H, *dd*, *J* = 14, 4 Hz, H-2′ax), 1.85 (1H, *dd*, *J* = 15, 4 Hz, H-4ax), 1.91 (1H, *dd*, *J* = 15, 4 Hz, H-2eq), 1.96 (3H, s, H-19′), 1.98 (3H, s, H-20′), 1.99 (6H, s, H-19 and H-20), 2.00 (1H, *dd*, not determined due to signal overlap, H-2′eq), 2.10 (1H, *dd*, *J* = 15, 4 Hz, H-4eq), 2.96 (1H, *dd*, *J* = 15, 9 Hz, H-4′eq), 4.28 (1H, t, *J* =3.5 Hz, H-3), 4.51 (1H, *m*, H-3′), 6.23 (1H, d, *J* =11 Hz, H-10), 6.28 (1H, d, *J* =11 Hz, H-14), 6.37 (1H, d, *J* =11 Hz, H-14′), 6.37 (2H, s, H-7 and H-8), 6.38 (1H, d, *J* =15 Hz, H-12), 6.45 (1H, d, *J* =15 Hz, H-7′), 6.53 (1H, d, *J* =15 Hz, H-12′), 6.56 (1H, d, *J* =11 Hz, H-10′), 6.62 (1H, *dd*, *J* = 11, 15 Hz, H-11′), 6.64 (1H, *m*, H-15′), 6.67 (1H, *dd*, *J* = 11, 15 Hz, H-11), 6.71 (1H, *m*, H-15), 7.33 (1H, d, *J* =15 Hz, H-8′).

Capsorubin (peak 21)

((*3S,5*R,3′*S*,5′R)-*3,3′-Dihydroxy-κ,κ-carotene-6,6′-dione*). Available amount: 5 mg. UV/VIS (EtOH, r.t., nm) 515 (sh), 484; (acetone) 507, 476; (*n*-hexane) 502, 468, 444. CD (EtOH, r.t., nm (mdeg)): 251 (-1.5), 302 (+3.0), 371 (-1.5). MALDI-MS, *m/z* = 600.4170 ± 0.0004 $[M]^+$, $C_{40}H_{56}O_4$ (9 mSigma). ESI-MS/MS, *m/z* (rel. int.): 685.80 ($C_{42}H_{62}O_6Na$, Calc. 685.4444, 12, $[M + Na+2CH_3O]^+$), 655.80 ($C_{41}H_{60}O_5Na$, Calc. 655.4338, 14, $[M + Na + CH_3OH]^+$), 623.75 ($C_{40}H_{56}O_4Na$, Calc. 623.4076, 35, $[M + Na]^+$), 601.60 ($C_{40}H_{57}O_4$, Calc. 601.4257, 100, $[M+H]^+$), 583.70 ($C_{40}H_{55}O_3$, Calc. 583.4151, 88, $[M+H-H_2O]^+$) ^{1}H-NMR δ (600 MHz, $CDCl_3$, including ^{1}H^{1}HCOSY, ^{1}H^{1}HNOESY): 0.84 (3H, s, H-16), 1.21 (3H, s, H-17), 1.37 (3H, s, H-18), 1.49 (1H, *dd*, *J* = 14, 3 Hz, H-4ax), 1.71 (1H, *dd*, *J* = 14, 5 Hz, H-2ax), 1.96 (3H, s, H-19), 1.99 (3H, s, H-20), 2.00 (1H, *dd*, *J* = 14, 8 Hz, H-2eq), 2.96 (1H, *dd*, *J* = 14, 9 Hz, H-4eq), 4.51 (1H, *m*, *J* = 4, H-3), 6.36 (1H, d, *J* =9 Hz, H-14), 6.45 (1H, d, *J* =15 Hz, H-7), 6.52 (1H, d, *J* = 15, H-12), 6.55 (1H, d, *J* = 12, H-10), 6.64 (1H, *dd*, *J* = 15, 12 Hz, H-11), 6.69 (1H, *dd*, *J* = 8, 3 Hz, H-15), 7.33 (1H, d, *J* =15 Hz, H-8). ^{13}C-NMR δ(150 MHz, $CDCl_3$, including DEPT-135, HSQC, HMBC): 12.8 (C20), 12.9 (C19), 21.3 (C18), 25.1 (C17), 25.9 (C16), 44.0 (C1), 45.2 (C4), 50.8 (C2), 59.0 (C5), 70.4 (C3), 121.1 (C7), 124.6 (C11), 131.2 (C15), 134.0 (C9), 134.9 (C14), 137.0 (C13), 140.5 (C10), 141.7 (C12), 146.8 (C8), 202.9 (C6).

Capsanthin (peak 31)

((*3*R,3′*S*,5′R)-*3,3′-Dihydroxy-β,κ-caroten-6′-one*). Available amount: 1 mg. UV/VIS (EtOH, r.t., nm) 476; (acetone) 470; (*n*-hexane) 497, 469. CD (EtOH, r.t., nm (mdeg)): 255 (+2.0), 297 (-2.9), 355 (+0.8). MALDI-MS, *m/z* = 584.4198 ± 0.0004 $[M]^+$, $C_{40}H_{56}O_3$ (14.6 mSigma). ESI-MS/MS, *m/z* (rel. int.): 669.75 ($C_{42}H_{62}O_5Na$, Calc. 669.4495, 30, $[M + Na+2CH_3O]^+$), 639.90 ($C_{41}H_{60}O_4Na$, Calc. 639.4389, 12, $[M + Na + CH_3OH]^+$), 615.65 ($C_{41}H_{59}O_4$, Calc. 615.4413, 32, $[M + CH_3O]^+$), 607.75 ($C_{40}H_{56}O_3Na$, Calc. 607.4127, 30, $[M + Na]^+$), 585.45 ($C_{40}H_{57}O_3$, Calc. 585.4308, 100, $[M+H]^+$), 567.70 ($C_{40}H_{55}O_2$, Calc. 567.4202, 21, $[M+H-H_2O]^+$). ^{1}H-NMR δ(500 MHz, $CDCl_3$): 0.84 (3H, s, H-16′), 1.08 (6H, s, H-16 and 17), 1.21 (3H, s, H-17′), 1.37 (3H, s, H-18′), 1.47 (1H, t, *J* =6 Hz, H-2ax), 1.49 (1H, *dd*, *J* = 15, 3 Hz, H-4′ax), 1.71 (1H, *dd*, *J* = 18, 4 Hz, H-2′ax), 1.74 (3H, s, H-18), 1.78 (1H, *dd*, *J* = 11, 3.5 Hz, H-2eq), 1.96 (3H, s, H-19′), 1.97 (6H, s, H-19 and 20), 1.99 (3H, s, H-20′), 2.00 (1H, t, *J* =7.5 Hz, H-2′eq), 2.05 (1H, *dd*, *J* = 15, 9 Hz, H-4ax), 2.39 (1H, *ddd*, *J* = 15, 5, 1.5, H-4eq), 2.96 (1H, *dd*, *J* = 15, 8, H-4′eq), ca. 4.00 (1H, br *m*, H-3), 4.52 (1H, *m*, H-3′), 6.13 (2H, s, H-7 and 8), 6.16 (1H, d, *J* =11 Hz, H-10), 6.26 (1H, d, *J* =11 Hz, H-14), 6.35 (1H, d, *J* =11 Hz, H-14′), 6.36 (1H, d, *J* = 15, H-12), 6.45 (1H, d, *J* = 15, H-7′), 6.52 (1H, d, *J* = 15, H-12′), 6.55 (1H, d, *J* = 11, H-10′), ca. 6.6–6.8 (4H, *m*, H-11,11′,1515′), 7.33 (1H, d, *J* =15 Hz, H-8′).^{13}C-NMR δ(125 MHz, $CDCl_3$): 12.8 (C19, C20), 12.9 (C19′, C20′), 21.3 (C18′), 21.6 (C18), 25.1 (C17′), 25.9 (C16′), 28.7 (C16), 30.3 (C17), 37.1 (C1), 42.6 (C4), 44.0 (C1′), 45.3 (C4′), 48.5 (C2), 50.9 (C2′), 58.9 (C5′), 65.1 (C3), 70.4 (C3′), 120.9 (C7′), 124.1 (C11′), 125.5 (C11), 125.9 (C7), 126.3 (C5), 129.7 (C15), 131.2 (C10), 131.6 (C15′), 132.4 (C14), 133.7 (C9′), 135.2 (C14′), 135.9 (C13′), 136.1 (C9), 137.4 (C12), 137.6 (C13), 137.7 (C6), 138.4 (C8), 140.7 (C10′), 141.9 (C12′), 146.8 (C8′), 202.9 (C6′).

Cryptocapsin (peak 52)

((3′*S*,5′R)-3′-*Hydroxy-β-κ-caroten*-6′-*one*). Available amount: ca. 3 mg. UV/VIS (EtOH, r.t., nm): 477; (acetone) 471; (*n*-hexane) 498, 471; (chloroform) 487. CD (EtOH, r.t., nm (mdeg)): 247 (-0.4), 292 (+1.2), 371 (-0.4). MALDI-MS, *m/z* = 568.4272 ± 0.0003 $[M]^+$, $C_{40}H_{56}O_2$ (12 mSigma). ESI-MS/MS, *m/z* (rel. int.): 623.90 ($C_{41}H_{60}O_3Na$, Calc. 623.4440, 22, $[M + Na + CH_3OH]^+$), 591.75 ($C_{40}H_{56}O_2Na$, Calc. 591.4178, 13, $[M + Na]^+$), 569.50 ($C_{40}H_{57}O_2$, Calc. 569.4359, 100, $[M+H]^+$), 551.75 ($C_{40}H_{55}O$, Calc. 551.4253, 15, $[M+H-H_2O]^+$). ^{1}H-NMR *δ* (600 MHz, $CDCl_3$, including ^{1}H^{1}HCOSY, ^{1}H^{1}HNOESY): 0.84 (3H, s, H-16′), 1.03 (6H, s, H-16 and H-17), 1.21 (3H, s, H-17′), 1.37 (3H, s, H-18′), 1.47 (2H, t, *J* =6 Hz, H-2), 1.49 (1H, *dd*, *J* = 16, 3 Hz, H-4′ax), 1.62 (2H, q, *J* =6 Hz, H-3), 1.71 (1H, *dd*, *J* = 13, 5 Hz, H-2′ax), 1.72 (3H, s, H-18), 1.96 (3H, s, H-19′), 1.98 (6H, s, H-19 and H-20), 1.99 (3H, s, H-20′), 2.00 (1H, *dd*, *J* = 13, 8 Hz, H-2′eq), 2.02 (2H, t, *J* =6 Hz, H-4), 2.96 (1H, *dd*, *J* = 14, 9 Hz, H-4′eq), 4.51 (1H, *m*, *J* =4 Hz, H-3′), 6.14 (1H, d, *J* =16 Hz, H-8), 6.15 (1H, d, *J* =11 Hz, H-10), 6.19 (1H, d, *J* =16 Hz, H-7), 6.26 (1H, d, *J* =12 Hz, H-14), 6.36 (2H, d, *J* =14 Hz, H-14′ and H-12), 6.44 (1H, d, *J* =15 Hz, H-7′), 6.52 (1H, d, *J* =15 Hz, H-12′), 6.55 (1H, d, *J* =11 Hz, H-10′), 6.61 (1H, *dd*, *J* = 15, 11 Hz, H-11′), 6.62 (1H, *dd*, *J* = 14, 12 Hz, H-15), 6.69 (1H, *dd*, *J* = 15, 11 Hz, H-11), 6.70 (1H, *dd*, *J* = 14, 11 Hz, H-15′), 7.33 (1H, *d*, *J* =15 Hz, H-8′). ^{13}C-NMR δ(150 MHz, $CDCl_3$, including DEPT-135, HSQC, HMBC): 12.7 (C19), 12.8 (C19′ and C20′), 12.9 (C20), 19.3 (C3), 21.3 (C18′), 21.8 (C18), 25.1 (C17′), 25.9 (C16′), 29.0 (C16 and C17), 33.1 (C4), 34.3 (C1), 39.6 (C2), 44.0 (C1′), 45.3 (C4′), 50.9 (C2′), 58.9 (C5′), 70.4 (C3′), 120.9 (C7′), 124.0 (C11′), 125.7 (C11), 127.0 (C7), 129.5 (C5 and C15), 130.7 (C10), 131.7 (C15′), 132.2 (C14), 133.6 (C9′), 135.3 (C14′), 135.8 (C13′ and C13), 136.5 (C9), 137.0 (C12), 137.7 (C6 and C8), 140.7 (C10′), 142.0 (C12′), 146.9 (C8′), 202.9 (C6′).

13-cis isomer of capsorubin (peak 29)

((*3S,5*R,3′*S*,5′R)-*3,3′-Dihydroxy-κ,κ-carotene-6,6′-dione*).

Available amount: 1 mg. UV/VIS (EtOH, r.t., nm) 374 (*cis* peak), 477 (Q- ratio = $A_{\lambda max\ cis\ peak}/A_{\lambda max\ main\ peak}$ = 0.47); (acetone) 368 (*cis* peak), 470, 500 (Q-ratio = 0.44); (*n*-hexane) 367 (*cis* peak), 439, 463, 496 (Q-ratio = 0.39). ESI-MS/MS, *m/z* (rel. int.): 685.65 ($C_{42}H_{62}O_6Na$, Calc. 685.4444, 12, $[M + Na+2CH_3O]^+$), 623.70 ($C_{40}H_{56}O_4Na$, Calc. 623.4076, 100, $[M + Na]^+$), 601.65 ($C_{40}H_{57}O_4$, Calc. 601.4257, 97, $[M+H]^+$), 583.70 ($C_{40}H_{55}O_3$, Calc. 583.4151, 75, $[M+H-H_2O]^+$).

3. Results

3.1. Separation of carotenoids in red fruit and red chili pepper by RP-HPLC

As shown in Fig. 2, 60 carotenoids were separated within 30 min from the hydrolyzed carotenoid extracts of red fruit and red chili pepper by RP-HPLC on a Cosmosil 5C18-MS-II column using gradient elution with a mixture of H_2O, MeOH, and acetone. As mentioned in the Section *2.3. Carotenoid separation*, this was the suboptimal work-up procedures to gain high resolution in the HPLC separation using a polar injection solvent. Accordingly, the different analytical system using the C30 column was also applied and compared with the C18 column, particularly

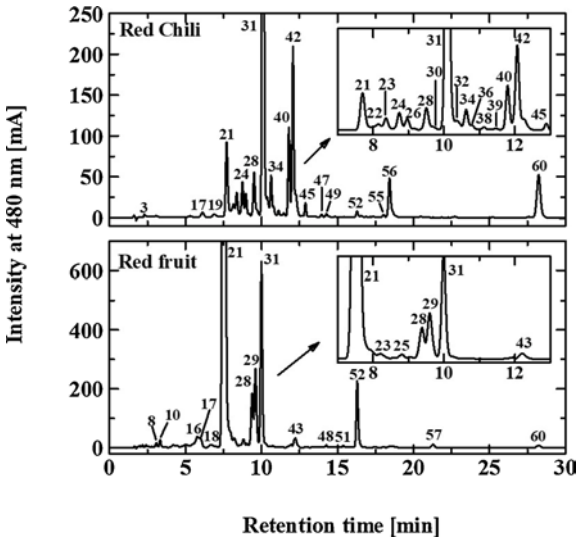

Fig. 2. Typical HPLC elution profiles of carotenoids in red fruit and red chili pepper detected at 480 nm. The separation of carotenoids was carried out by RP-HPLC using a Cosmosil 5C18-MS-II column and an elution gradient of a mixture of H_2O, MeOH and acetone. The peak numbers in the elution profile correspond to those in Tables 1 and S1.

for xanthophyll cycle carotenoids.

In red fruit, 36 peaks were separated; among them, 13 carotenoids were identified. However, in red chili pepper, 19 carotenoids were identified out of 37 separated peaks. The summary of the assigned carotenoids and their MS results in red fruit and red chili pepper is shown in Table 1, while the detailed data of the separation and identification is presented in Table S1. To understand more clearly, particularly quantification, a simplified Table only for the assigned carotenoids and their relative contents is also shown in Table S2.

3.2. Structural identification of main carotenoids from red fruit

The structures of carotenoids with the peaks 17, 21, 31, and 52 were unequivocally determined by our spectroscopic methods to be 5,6-diepicapsokarpoxanthin, capsorubin, capsanthin, and cryptocapsin, respectively. All the ^{1}H-NMR signals for peak 17 were assigned, and the chemical shifts were consistent with those of 5,6-diepicapsokarpoxanthin reported by Deli et al. (1998) within 0.01 ppm. The presence of three hydroxy groups at the 3, 3′ and 5 positions was confirmed by ESI-TOF-MS fragment peaks at *m/z* 601.4 ($[M+H–H_2O]^+$), 583.4 ($[M+H–2H_2O]^+$), and 565.4 ($[M+H–3H_2O]^+$). The configurations of the hydroxy groups at the 3, 5, and 6 positions were unequivocally determined to be (3*S*,5*S*,6*S*) in peak 17 by comparing the ^{1}H-NMR chemical shifts of the corresponding protons; i.e., H-16 (δ 0.84), H-17 (δ 1.31), H-2ax (δ 1.65), H-4ax (δ 1.84), H-2eq (δ 1.90), H-4eq (δ 2.10), H-7 (δ 6.36) and H-8 (δ 6.36) (Deli et al., 1998).

For peaks 21, 31, and 52, all the ^{1}H-NMR signals were unequivocally assigned, and the chemical shifts were identical to those listed in Rüttimann et al. (1983) and Englert (1995). The chemical formulae were determined by high-resolution MS. The CD spectra of peaks 21 and 52 show similar cotton effects of 251 (–), 302 (+), and 371 (–) for the former, and 247 (–), 292 (+), and 371 (–) for the latter, whereas the CD spectra of peak 31 show an opposite pattern of 255 (+), 297 (–), and 355 (+). These findings were consistent with the configurations of (3*S*,5*R*, 3′*S*,5′*R*) and (3′*S*,5′*R*) for peaks 21 and 52, respectively, and (3*R*,3′*S*,5′*R*) for peak 31. The spectral data confirmed the assignments of peaks 21, 31, and 52 as capsorubin, capsanthin, and cryptocapsin, respectively.

Assignment of the carotenoids was also confirmed by the results of ESI-MS/MS measurement, and the MS spectra obtained are shown in Fig. 3. 5,6-Diepicapsokarpoxanthin ($C_{40}H_{58}O_5$) had a precursor ion at *m/z* 619.55 $[M+H]^+$ and product ions at *m/z* 601.60 $[M+H–H_2O]^+$ and *m/z* 583.65 $[M+H–2H_2O]^+$. The mass spectra of capsorubin ($C_{40}H_{56}O_4$), capsanthin ($C_{40}H_{56}O_3$), and cryptocapsin ($C_{40}H_{56}O_2$) showed the protonated molecular mass and fragment ion at *m/z* 601.60 $[M+H]^+$ and *m/z* 583.70 $[M+H–H_2O]^+$, at *m/z* 585.45 $[M+H]^+$ and *m/z* 567.70 $[M+H–H_2O]^+$, at *m/z* 569.50 $[M+H]^+$ and *m/z* 551.75 $[M+H–H_2O]^+$, respectively. The chemical structure of *κ*-cyclic carotenoids found in this study is shown in Figure S1. The other results of absorption, FT-IR, and CD spectroscopies are presented in Supporting information, Figures S2 to S4, respectively, and Table S4 for the summary of FT-IR results.

The other purified major carotenoid, peak 29, was determined to be the 13-*cis* isomer of capsorubin according to the results of MS analysis and cochromatography between the carotenoid extract of red fruit and the purified 13-*cis* isomer of capsorubin prepared by thermal-induced stereomutation of the purified capsorubin, in addition to the absorption peak ratio, the Q-ratio (ratio of the height of the *cis*-peak to the main absorption peak) (Figure S5 and Table S5). The 13-*cis* isomer of capsorubin ($C_{40}H_{56}O_4$) had a precursor ion at *m/z* 601.65 $[M+H]^+$ and a product ion at *m/z* 583.70 $[M+H–H_2O]^+$ (Fig. 3).

3.3. Identification of capsanthin epoxide group from red fruit

Peaks 23, 27, and 28 were determined to be capsanthin 5,6-epoxide, capsanthin 5,8-epoxide, and capsanthin 3,6-epoxide, respectively, by MS analysis and compared with the carotenoids in red chili pepper (Deli et al., 2001). They had the same protonated molecular mass at *m/z* 601.50–601.60 $[M+H]^+$ and product ion at *m/z* 583.40–583.50 $[M+H–H_2O]^+$, coinciding with those of the capsanthin epoxide groups (Figure S6). Capsanthin 5,6-epoxide (peak 23) was confirmed by an epoxide test that gave a blueshift of an absorption peak at 454 nm by adding acid (Eugster, 1995). Simultaneously, its product, capsanthin 5, 8-epoxide, was also confirmed to be peak 27 by the emerging new peak with the same t_R. The latter two epoxides, capsanthin 5,8-epoxide, and capsanthin 3,6-epoxide, were epoxide test negative, coinciding with their property.

3.4. Comparison of the carotenoids between red fruit and red chili pepper

In the fruit of red chili pepper, 12 carotenoids that have identical t_R to red fruit were identified parallel to the red fruit carotenoids during their characterizations. Seven other carotenoids were identified according to the spectral shape, λ_{max} and order of elution profile compared with the previous carotenoid assignment by Deli et al. (2001) as follows: peak 24 = 5,6-diepikarpoxanthin; peak 26 = violaxanthin; peak 36 = antheraxanthin; peak 38 = *8R* mutatoxanthin; peak 39 = *8S* mutatoxanthin; peak 40 = lutein; and peak 42 = zeaxanthin. These identifications were also confirmed by ESI-MS/MS analyses using highly purified samples from the fruit of red chili pepper as shown in Table 1 and Figure S7 (A-C)—. They were all xanthophyll species without a *κ*-ring structure. Accordingly, among 13 carotenoids identified in red fruit, 12 carotenoids were common to those of red chili pepper, and 8, including the *cis*-isomer, were *κ*-cyclic carotenoids that are characteristic in the red fruit of *Capsicum* plants. Furthermore, the contents estimated by the relative peak area were quite high in both plants (Tables 1 and S2). In red fruit, the capsorubin contents were the highest at 71.2 %, followed by the capsanthin content at 9.65 % of the total, whereas capsanthin was the highest at 56.7 % and capsorubin was 2.99 % in red chili pepper. The concentrations of capsorubin and capsanthin per g of dry weight in red fruit were estimated as 2719 ± 60.21 μg and 305.0 ± 97.06 μg (mean ± SE, n = 3), respectively, from their standard curves, while those in red chili pepper were 97.47 ± 18.97 μg and 1344 ± 239.8 μg, respectively. The concentrations in red chili pepper were comparable to those reported by Arimboor et al. (2015). These results indicate high similarity not only in their carotenoid compositions but also in their concentrations between both plant species. Unlike red chili pepper, 5,6-diepikarpoxanthin, mutatoxanthins, and xanthophyll cycle carotenoids, such as

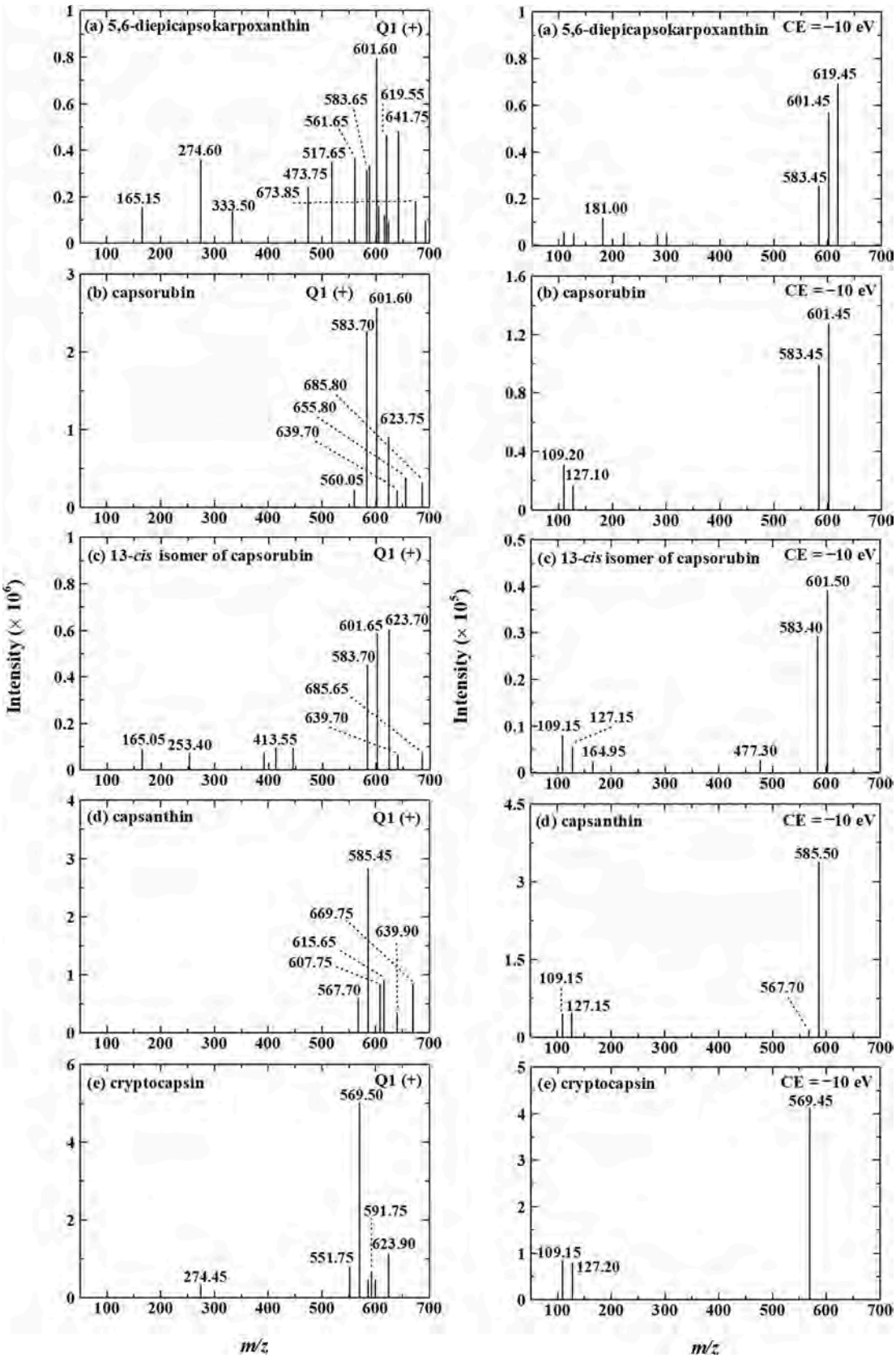

Fig. 3. Typical ESI-MS/MS spectra of the purified carotenoids from red fruit: (a) 5,6-diepicapsokarpoxanthin; (b) capsorubin; (c) 13-*cis* isomer of capsorubin; (d) capsanthin; and (e) cryptocapsin analyzed by LC-MS/MS using the modes of Q1 scan (+) (left) and product ion scan (CE =−10 eV) (right). The analytical conditions are described in the text.

zeaxanthin, antheraxanthin, and violaxanthin, as well as lutein were not detected in red fruit (Table 1). To confirm the absence of those carotenoids, particularly for xanthophyll cycle carotenoids, a different analytical system using the C30 column was applied and compared with the C18 column. Additionally, a high concentration of the sample was used to avoid the detection limit due to a low sample concentration as mentioned in the Section *2.3*. The results showed that peaks with a similar t_R to the target carotenoids were found in both columns (Fig. 4, A). However, their t_R values did not completely correspond to those of the standards, and their spectra were different from their respective standards (Fig. 4, B). The t_R and λ_{max} of the peak groups shown in Fig. 4 are summarized in Table S3. Thus, it is likely that they were not present in red fruit.-

Peak 54 was a trace component with content of only 0.01 % in red fruit and 0.02 % in red chili pepper. Due to elaborate analyses by spectroscopy and ESI-LCMS-MS, this peak was finally determined to be *β*-cryptoxanthin 5,6-epoxide with the precursor ion at *m/z* 568.30 $[M]^+$ and fragment ion at *m/z* 560.25 $[M–H_2O]^+$. In both plant species, peaks 55, 56, 59, and 60 were identified as *α*- and *β*-cryptoxanthin and *α*- and *β*-carotene, respectively. These results agree with the findings in previous reports (Deli et al., 2001; Sarungallo et al., 2015b). In red fruit, the carotenoids, including those with the 3-hydroxy-*β*-end group, except for capsanthin, and others were minor species with a relative content of only 0.25 %, in contrast to 31.1 % in red chili pepper (Table 1, see also Table S2).

4. Discussion

In the present study, the five purified carotenoids in red fruit were unequivocally identified by spectroscopic and/or chromatographic methods as 5,6-diepicapsokarpoxanthin, capsorubin, capsanthin, cryptocapsin, and 13-*cis* isomer of capsorubin (Table 1; Tables S4–S5; Fig. 3; Figures S2–S5). In addition to those, three capsanthin epoxides, *β*-cryptoxanthin 5,6-epoxide and other precursors such as *α*-, *β*-cryptoxanthins, and *α*-, *β*-carotenes were assigned (Table 1). A similar result was also obtained from a short syncarp cultivar of red fruit. Despite largely different taxa of monocots and dicots, the same 7 carotenoids, except for *cis*-isomer, of *κ*-cyclic carotenoids were shared between red fruit and red chili pepper (Tables 1 and S2; Figure S1 for chemical structure). Thus, *κ*-cyclic carotenoids are no longer unique in *Capsicum* plants, although only a few species of *κ*-cyclic carotenoids have been found in different plant species to date (Bubicz, 1965; Chacón-ordóñez et al., 2017; Deli et al., 2000; Jeknić et al., 2012; Murillo et al., 2013). The *κ*-cyclic carotenoid contents in red fruit accounts for 91.68 % of the total 36 different structures of carotenoids as estimated by the peak area, indicating that carotenoids in red fruit are composed mostly of *κ*-cyclic

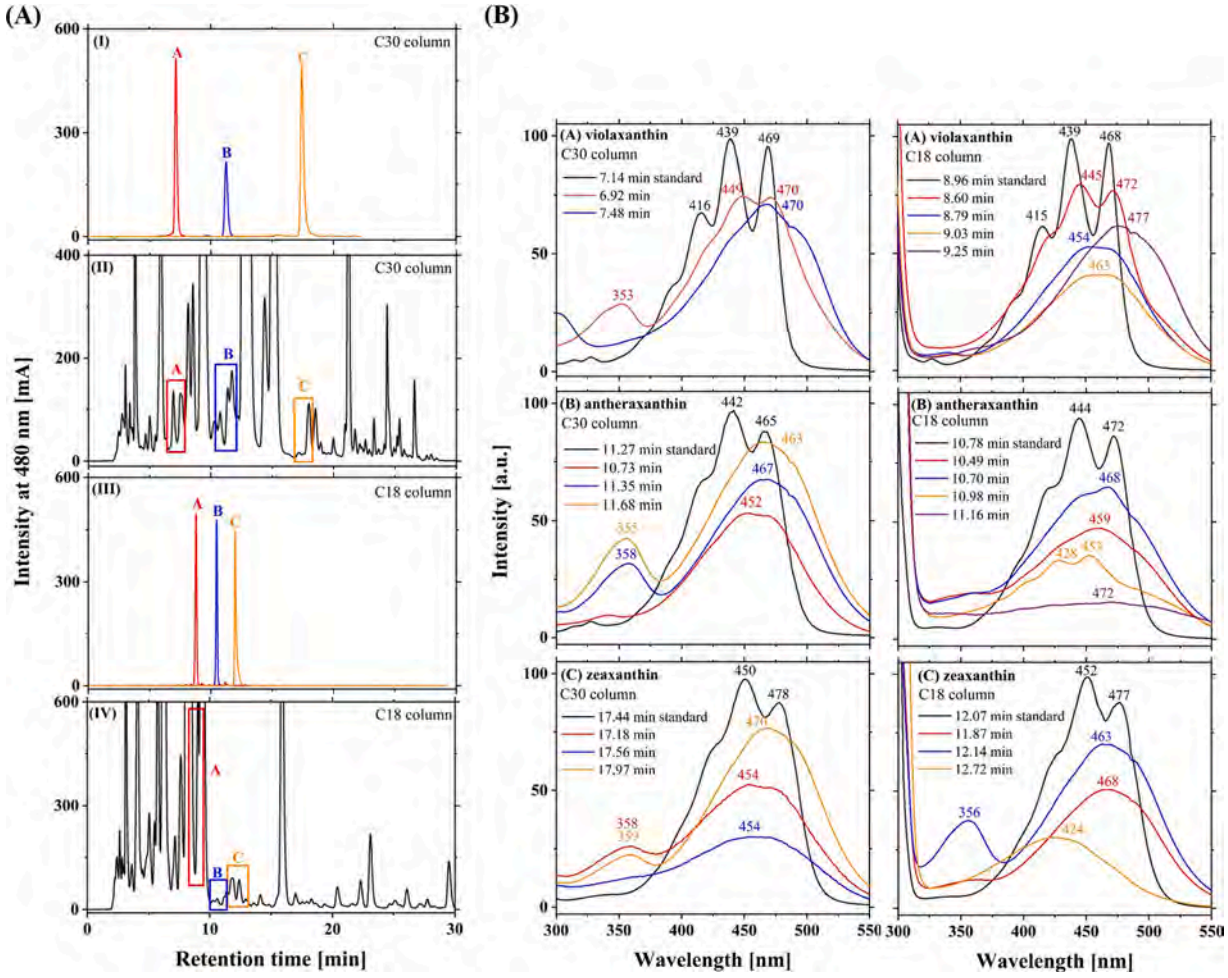

Fig. 4. (a). HPLC analysis of the standards and carotenoid extracts from red fruit using the C30 and C18 columns and a high concentration of the sample. The peak groups with similar t_R to the respective standards were selected as possible candidates for the xanthophyll cycle carotenoids. The standards and their peak groups are violaxanthin (A, red), antheraxanthin (B, blue), and zeaxanthin (C, gray). HPLC chromatograms detected at 480 nm: (I) and (III), standard carotenoids of the peak groups and (II) and (IV), carotenoid extracts, separated by the C30 and C18 columns, respectively.
(b). In-line absorption spectra of the peak groups shown in Fig. 4, A. The peak groups are (A) violaxanthin; (B) antheraxanthin; and (C) zeaxanthin. The intensity is normalized to 100 in the standard.

species compared with 63.81 % of the carotenoids in red chili pepper. However, the contents of carotenoids belonging to the 3-hydroxy-β-end group and others in red fruit were quite low compared with those in red chili pepper. Accordingly, not only the composition but also the quantitation of the carotenoids in red fruit is comparable to that of red chili pepper. Unlike the fruit of red chili pepper, however, red fruit lacks xanthophyll (violaxanthin) cycle carotenoids and lutein (Table 1 and Figs. 2 and 4).

The red fractions containing κ-cyclic carotenoids were identified from the present species. Additionally, this is the second report on 5,6-diepicapsokarpoxanthin isolated from a natural source and, to our best knowledge, the first report from non-*Capsicum* species. The biosynthetic precursor of the 3,5,6-trihydroxy end group (3,5,6-trihydroxy-5,6-dihydro-β-end group) has been proposed to be the 3-hydroxy-5,6-epoxy-β-ionone end group (3-hydroxy-5,6-epoxy-β-end group) (Deli et al., 1998). Accumulation of the specific (3*S*,5*S*,6*S*) configuration suggests that this is not an artifact produced by the pinacol rearrangement. This pigment is reported to be produced from capsanthin 5,6-epoxide by flexible stabilization of incipient carbocation generated by capsanthin-capsorubin synthetase (Mialoundama et al., 2010).

The biosynthetic pathway of κ-cyclic carotenoids has been previously proposed (Davies et al., 1970; Deli et al., 2000; Mialoundama et al., 2010), with two main biosynthetic routes from β-cryptoxanthin and zeaxanthin. A recent study on the orange and orange-red color tepals of the tiger lily showed that two κ-cyclic carotenoids, capsanthin and capsorubin, were accumulated and produced from antheraxanthin and violaxanthin, respectively (Jeknić et al., 2012), similar to *Asparagus* (Deli et al., 2000). In the present study, however, we could not detect any such precursors, not only antheraxanthin and violaxanthin but also their precursor zeaxanthin (Fig. 2 and Table 1). These findings were confirmed in parallel by the analysis using the C30 column and a high concentration of the samples (Fig. 4 and Table S3). Furthermore, the absence of antheraxanthin is partly supported by 5,6-diepikarpoxanthin, a byproduct of capsanthin biosynthesis from antheraxanthin catalyzed by the enzyme capsanthin-capsorubin synthetase (Mialoundama et al., 2010), could not be detected in red fruit unlike red chili pepper (Table 1). Therefore, a route other than the precursors derived from zeaxanthin may be involved in the biosynthesis of κ-cyclic carotenoids in red fruit. Further understanding of the regulation mechanism of the intermediates β-cryptoxanthin and zeaxanthin in the biosynthetic pathway of κ-cyclic carotenoids is necessary.

5. Conclusion

The carotenoid composition from the monocot *Pandanus* plant of the Papua Islands, red fruit (*P. conoideus*), was investigated by chromatographic and spectrometric analyses. Eight κ-end group carotenoids, capsorubin, capsanthin, cryptocapsin, 13-*cis* capsorubin, 5,6-diepicapsokarpoxanthin, and three capsanthin epoxides were separated and identified from red fruits. Despite the large taxonomical difference between red fruit and red chili pepper, a high similarity in the composition and contents of the κ-end group carotenoids was found in both plants. These findings provide useful information and help better understand the structural diversity of the carotenoids in different plant taxa.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary material files.

Code availability

All data in this study are included in this published article and its supplementary material files.

Ethics approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Informed consent

There is no clinical trial related to any patients in this study.

Author's contributions

THPB conceived and designed the experiments, funding acquisition, writing original draft and approved the final draft. H designed and performed the experiments for extraction, chromatography, FT-IR and MS/MS measurement, as well as prepared figures and/or tables, and writing original draft. IAG performed the experiments for extraction and chromatography. RF designed experiments for HRMS and CD spectroscopy as well as analyzed data. TM designed experiments for NMR and analyzed data. YS designed the experiments, prepared and developed the original draft. LL and KMBK designed sampling, sample preparation and molecular genetics analysis. LL acquired part of fundings.

Fundings

We acknowledge the funding from the Directorate of Research and Community Services, Ministry of Research and Technology/National Research and Innovation Agency of the Republic of Indonesia under the Fundamental Research scheme (grant number 005/MACHUNG/LPPM/SP2H-LIT-MULTI/AMD/VI/2020) and the World Class Research scheme (grant number 007/MACHUNG/LPPM/SP2H-LIT-MULTI/AMD/VI/2020) acquired by THPB for the support in experiments, i.e. extraction, separation/purification, analysis of carotenoids, and publication. We also acknowledge the Fundamental Research scheme (grant number 005/PER-P2M/UPJ-DIKTI/04.19) acquired by LL for the support in material sampling and sample preparation.

Declaration of Competing Interest

None of the authors have conflicts of interest to declare.

Acknowledgements

The authors thank to Mr. Leonardo Elisa Aisoi, Biology Education Department, Faculty of Teacher Training and Education, Cenderawasih University, for collecting red fruit. RF thanks Ms. Tomomi Shimonaka and Dr. Matsumi Doe at Analysis Division, Research Center for Artificial Photosynthesis, Osaka City University for measuring HRMS and NMR (600 MHz) spectra, respectively. RF also thanks Dr. Nami Yamano and Mr. Soichiro Seki for measuring CD spectra.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jfca.2020.103722.

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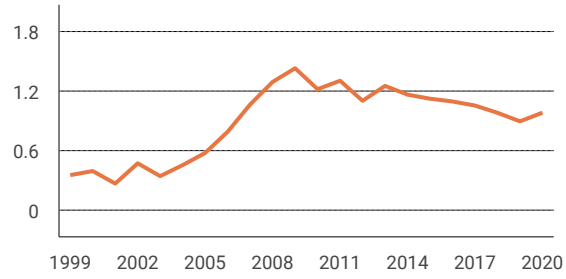

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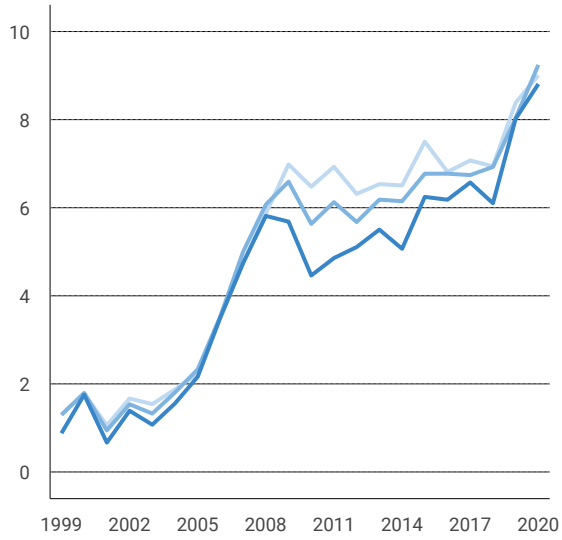

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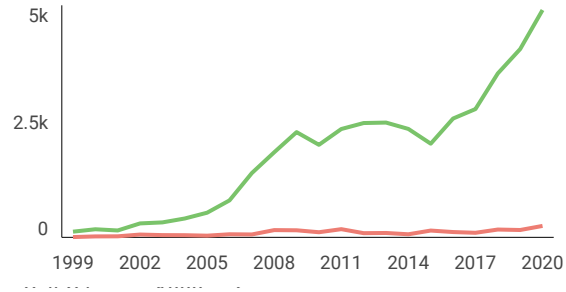

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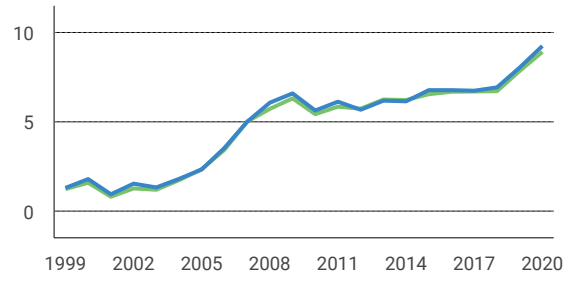

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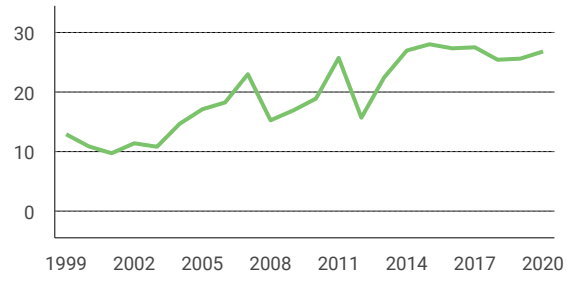

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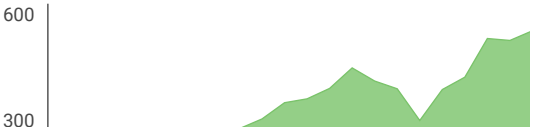

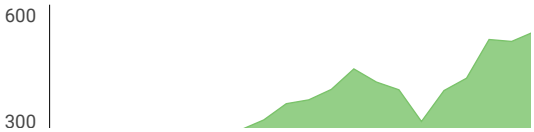

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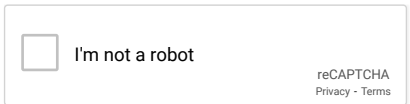

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Von:	Journal of Food Composition and Analysis
An:	Tatas H.P Brotosudarmo, Ph.d
Betreff:	Successfully received: submission Carotenoids from Buah Merah (Pandanus conoideus), an Indigenous Red Fruit of Papua: Compositional Similarity to the Fruit of Red Chili Pepper (Capsicum annuum) for Journal of Food Composition and Analysis
Datum:	Samstag, 25. April 2020 03:33:59

This message was sent automatically.

Ref: JFCA_2020_660
Title: Carotenoids from Buah Merah (Pandanus conoideus), an Indigenous Red Fruit of Papua: Compositional Similarity to the Fruit of Red Chili Pepper (Capsicum annuum)
Journal: Journal of Food Composition and Analysis

Dear Dr. Brotosudarmo,

Thank you for submitting your manuscript for consideration for publication in Journal of Food Composition and Analysis. Your submission was received in good order.

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Journal of Food Composition and Analysis

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Von:	Journal of Food Composition and Analysis
An:	Tatas H.P Brotosudarmo, Ph.d
Betreff:	Your manuscript JFCA_2020_660 has been sent for review
Datum:	Samstag, 9. Mai 2020 23:30:29

This message was sent automatically.

Reference: JFCA_2020_660
Title: Carotenoids from Buah Merah (Pandanus conoideus), an Indigenous Red Fruit of Papua: Compositional Similarity to the Fruit of Red Chili Pepper (Capsicum annuum)
Journal: Journal of Food Composition and Analysis

Dear Dr. Brotosudarmo,

I am currently identifying and contacting reviewers who are acknowledged experts in the field. Since peer review is a voluntary service it can take time to find reviewers who are both qualified and available. While reviewers are being contacted, the status of your manuscript will appear in EVISE® as 'Reviewer Invited'.

Once a reviewer agrees to review your manuscript, the status will change to 'Under Review'. When I have received the required number of expert reviews, the status will change to 'Ready for Decision' while I evaluate the reviews before making a decision on your manuscript.

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Journal of Food Composition and Analysis

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Von:	Journal of Food Composition and Analysis
An:	Tatas H.P Brotosudarmo, Ph.d
Betreff:	Reviews complete and decision pending for your manuscript JFCA_2020_660
Datum:	Montag, 11. Mai 2020 14:38:32

Reference: JFCA_2020_660
Title: Carotenoids from Buah Merah (Pandanus conoideus), an Indigenous Red Fruit of Papua: Compositional Similarity to the Fruit of Red Chili Pepper (Capsicum annuum)
Journal: Journal of Food Composition and Analysis

Dear Dr. Brotosudarmo,

I am pleased to inform you that I have received all the required reviews, which I will now evaluate before making a decision on your manuscript referenced above.

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Thank you again for submitting your manuscript to Journal of Food Composition and Analysis and for giving me the opportunity to consider your work.

Kind regards,

Journal of Food Composition and Analysis

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Von:	Journal of Food Composition and Analysis
An:	Tatas H.P Brotosudarmo, Ph.d
Betreff:	Reviews complete and decision pending for your manuscript JFCA_2020_660
Datum:	Dienstag, 9. Juni 2020 04:44:43

Reference: JFCA_2020_660
Title: Carotenoids from Buah Merah (Pandanus conoideus), an Indigenous Red Fruit of Papua: Compositional Similarity to the Fruit of Red Chili Pepper (Capsicum annuum)
Journal: Journal of Food Composition and Analysis

Dear Dr. Brotosudarmo,

I am pleased to inform you that I have received all the required reviews, which I will now evaluate before making a decision on your manuscript referenced above.

In the event that I need to seek the opinion of an additional reviewer, you may see the status of your manuscript revert briefly from 'Ready for Decision' to 'Under Review'.

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I will inform you once I have made a decision.

Thank you again for submitting your manuscript to Journal of Food Composition and Analysis and for giving me the opportunity to consider your work.

Kind regards,

Journal of Food Composition and Analysis

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Von: Journal of Food Composition and Analysis
An: Tatas H.P Brotosudarmo, Ph.d
Betreff: Re: Missing Supporting Information for JFCA_2020_660R1 [200914-007871]
Datum: Montag, 14. September 2020 18:43:01

Dear Dr. Brotosudarmo,

Thank you for providing the requested files and I have sent them to the Reviewers.

Warm regards,

Asogan Magendran
Journal Manager
Tel: +91 44 3378 4301

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From: Tatas Brotosudarmo
Date: Monday, September 14, 2020 10:33 AM GMT

Dear Dr. Magendran,

Herewith I send you the supporting information that was missing in our pdf submission.

Tatas H.P. Brotosudarmo, Dipl.Chem., Ph.D.
PI Chromatography and Spectroscopy Analysis of Biomaterials
MA CHUNG RESEARCH CENTER FOR PHOTOSYNTHETIC PIGMENTS
Registered as a National Center of Excellence (RTHE Minister Decree No: 11/M/Kp/II/2015)
Universitas Ma Chung; Villa Puncak Tidar N-01; Malang 65151 Jawa Timur; Indonesia
T:+62 341 550 171 ext. 4310 ; F:+62 341 550 175
Website: http://mrcpp-people.machung.ac.id/brotosudarmo-thp/home

---

Vice-President for Science and Policy of the Indonesian Young Academy of Sciences, 2018-2020
Fellow of the Alexander von Humboldt, Department of Experimental Physics, University of Bayreuth, 2020-2022
Board of Trustees of the Indonesian Chemical Society, 2020

Re: Missing Supporting Information for JFCA_2020_660R1 [200914-007871]

From: Asogan Magendran
Date: Monday, September 14, 2020 09:24 AM GMT

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In the manuscript document, you have mentioned a new Figure S7 to be found in the Online Supplementary Information. However, this information has not been included in the submission PDF. Can you check if the you have forgotten to upload this or if it got "lost" somehow in the system?

Kindly send the Supplementary files as early as possible to resume the review process.

Warm regards,

Asogan Magendran
Journal Manager
Tel: +91 44 3378 4301

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From: Asogan Magendran
Date: Monday, September 14, 2020 09:21 AM GMT

Manuscript Number: JFCA_2020_660R1
Carotenoids from Buah Merah (Pandanus conoideus), an Indigenous Red Fruit of Papua: Compositional Similarity to the Fruit of Red Chili Pepper (Capsicum annuum)
Journal of Food Composition and Analysis

Von:	Journal of Food Composition and Analysis
An:	Tatas H.P Brotosudarmo, Ph.d
Betreff:	Re: Missing Supporting Information for JFCA_2020_660R1 [200914-007871]
Datum:	Montag, 14. September 2020 11:25:03

Dear Dr. Brotosudarmo,

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Warm regards,

Asogan Magendran
Journal Manager
Tel: +91 44 3378 4301

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From: Asogan Magendran
Date: Monday, September 14, 2020 09:21 AM GMT

Manuscript Number: JFCA_2020_660R1
Carotenoids from Buah Merah (Pandanus conoideus), an Indigenous Red Fruit of Papua: Compositional Similarity to the Fruit of Red Chili Pepper (Capsicum annuum)
Journal of Food Composition and Analysis

Von:	em.jfca.0.6f46c5.626b9b2f@editorialmanager.com im Auftrag von Journal of Food Composition and Analysis
An:	Tatas H.P Brotosudarmo, Ph.d
Betreff:	Decision on submission to Journal of Food Composition and Analysis
Datum:	Donnerstag, 12. November 2020 23:02:43

Manuscript Number: JFCA_2020_660R2

Carotenoid composition in buah merah (Pandanus conoideus Lam.), an indigenous red fruit of the Papua Islands

Dear Dr Brotosudarmo,

Thank you for submitting your manuscript to Journal of Food Composition and Analysis.

I am pleased to inform you that your manuscript has been accepted for publication.

My comments, and any reviewer comments, are below.
Your accepted manuscript will now be transferred to our production department. We will create a proof which you will be asked to check, and you will also be asked to complete a number of online forms required for publication. If we need additional information from you during the production process, we will contact you directly.

We appreciate you submitting your manuscript to Journal of Food Composition and Analysis and hope you will consider us again for future submissions.

Kind regards,
Katherine Phillips
Handling Editor

Journal of Food Composition and Analysis

Editor and Reviewer comments:

Reviewer #1: The authors have adequately described the potential limitations and have improved all other instances according to my comments. I congratulate the authors and believe that the papers can now be accepted for publication in JFCA.

Reviewer #3: The manuscript can be published in the Journal of food composition and analysis in its present form.

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Revision requested for JFCA_2020_660

Katherine Phillips (Journal of Food Composition and Analysis)
<EviseSupport@elsevier.com>
Sun 12/07/2020 5:25 AM
To: Tatas H.P Brotosudarmo, Ph.d <tatas.brotosudarmo@machung.ac.id>

Ref: JFCA_2020_660
Title: Carotenoids from Buah Merah (Pandanus conoideus), an Indigenous Red Fruit of Papua: Compositional Similarity to the Fruit of Red Chili Pepper (Capsicum annuum)
Journal: Journal of Food Composition and Analysis

Dear Dr. Brotosudarmo,

Thank you for submitting your manuscript to Journal of Food Composition and Analysis. I have completed the review of your manuscript and a summary is appended below. The reviewers recommend reconsideration of your paper following major revision. I invite you to resubmit your manuscript after addressing all reviewer comments.

When resubmitting your manuscript, please carefully consider all issues mentioned in the reviewers' comments, outline every change made point by point, and provide suitable rebuttals for any comments not addressed.

PLEASE CAREFULLY READ THE FOLLOWING INSTRUCTIONS BEFORE BEGINNING REVISIONS:

I. Responding to reviewer and editor comments and questions: Beneath my signature, you will find a list of comments, questions, criticisms and suggestions from the reviewers and me, **along with the list entitled "Requirements Concerning All JFCA Submissions**". Copy the entire list of comments from reviewers and editor **and the "Requirements Concerning All JFCA Submissions"** into a separate file to be saved as "Detailed Response to Reviewers", and address each comment directly underneath. You MUST also provide the edits made and the line numbers (in the revised manuscript) where each revision occurs. (Line numbers may change between the version that was reviewed and your revision; be sure to indicate the NEW line numbers in your responses to each reviewer/editor query.) If you choose not to make a change that is suggested by a reviewer or the editor, you must provide a substantive reason for not doing so. You must also indicate for each item in the Requirements concerning all JFCA submisssions that you have thoroughly checked the manuscript submission for each point, and indicate "checked and no changes needed" or list the changes made and line numbers where they occur for each change.

II. Revising your paper: If this is the first revision of the paper, proceed to make the revisions, in TRACKED CHANGES (please also see point #1 in the Requirements list concerning tracked changes, below), on the copy of your manuscript returned to you through the EVISE system.

III. Resubmitting your revised paper: The revised paper in TRACKED CHANGES, and with the detailed responses to reviewers as outlined above, must be re-uploaded into the online system within 30 days. You do not need to include a "clean" version with "all changes accepted"; the editors and reviewers are accustomed to reading revised papers that show all changes made.

To submit your revised manuscript:

- Log into EVISE® at: http://www.evise.com/evise/faces/pages/navigation/NavController.jspx?JRNL_ACR=JFCA
- Locate your manuscript under the header 'My Submissions that need Revisions' on your 'My Author Tasks' view
- Click on 'Agree to Revise'
- Make the required edits
- Click on 'Complete Submission' to approve

What happens next?

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I look forward to receiving your revised manuscript as soon as possible.

Kind regards,

Dr. Phillips
Handling Editor
Journal of Food Composition and Analysis

Comments from the editors and reviewers:
-**Editor**

-

-Abstract is too short and needs quantitative data.
-Sampling plan needs to be described and justified.
- Title might need to be changed to indicate this isn't a quantitative comparison but showing similar carotenoids. Is this more of a short communication?
-**VERY IMPORTANT: Reviewer 1 raised significant concerns about analytical methods. These comments must be THOROUGHLY and CAREFULLY addressed, in detail (both in substance of the work and with revision to the text that address these questions/comments.**
- add more keywords
- Some highlights are too long, and need to be revised to comply with journal requirements (SEE GUIDELINES FOR AUTHORS)
- Please use the reference citation style and reference list format and style as specified in GUIDELINES FOR AUTHORS
- add section numbers
- the conclusion should be a separate section
- Please remove author names from supplementary material

-**Reviewer 1**

-

The authors presented a manuscript about the compositional similarity of the carotenoid profiles of the fruit of *Pandanus conoideus* (Buah Merah) and red chili

peppers.

The article claims to be first report about the carotenoid profile of the red colored fruit of *P. conoideus*, which is used by natives of Papua Island to color their food. The research topic is definitely interesting due to the on-going search of the food industry for new red plant pigments. However, the manuscript contains several analytical drawbacks. My main concern is about compound identification.

- The authors extracted the carotenoids with an apolar 1:1 mixture of ethanol and hexane (Line 72-73), but then apparently make up the extract in comparably polar methanol (Line 95). I am afraid that apolar carotenoids like carotenes (e.g., lycopene) insoluble or only poorly soluble in methanol might have been overlooked by the authors. Why did the authors decide to do it like this?

- Using C30 columns is state-of-the-art in carotenoid analyses, particularly for the separation of isomers. Why did the authors choose to use a C18 column on their analytical HPLC, but a C30 on their preparative LC? I am afraid that the purity checks apparently needed for MALDI and ESI-ToF-MS might have been insufficent therefore. Minor impurities might have caused potential MALDI mass signals. Please also see next comment.

- The data regarding the identification of most compounds as shown in Table 1 and Table S1 is insufficient. It has been carried out solely by comparing of UV/Vis spectra and retention times to those given in a previous report (Deli et al., 2001), except for 5 compounds they have isolated and characterized in more detail (capsorubin, 13-*cis*-capsorubin, capsanthin, cryptocapsin, 5,6-diepicapsokarpoxanthin,). It is unclear why the authors did the effort of characterizing these compounds in this tedious way of isolating by preparative LC etc.; Most of them are commercially available as standards, except for maybe the 5,6-diepicapsokarpoxanthin. Why did the authors not simply use their LC-MS/MSnsystem to provide mass data for ALL compounds detected? None of the 5 isolated compounds were novel and the instrumentation would have easily sufficed to characterize them with certainty, along with probably most of the other carotenoids. Currently, ca. 35 of the 60 compounds reported in Table S1 remain completely unknown and only the UV/Vis spectra are given. MS data are lacking completely. It is unclear how other assigned compounds (like alpha- and beta-cryptoxanthin) were identified? How did the authors, for instance, differentiate alpha-cryptoxanthin from its potential isomer zeinoxanthin?

- Therefore, I also have doubts that antheraxanthin and violaxanthin were identified properly in the red chili pepper. In my view, its impossible to do that simply with UV/Vis spectra and retention time orders taken from a paper that described the profile with a different HPLC system. Nevertheless, the authors used the chili pepper chromatogram then as a cross-reference to confirm the apparent absence of these two compounds in *P. conoideus* fruit too. Since antheraxanthin and violaxanthin are also part of the photosynthetic apparatus, they might also occur in a plant organ without being utilized for keto-carotenoid synthesis. In brief, when looking at the current data, the authors

cannot make these strong statements about the biosynthetic origin of the keto-carotenoids in this fruit, in my opinion.

- I am also having doubts about the LC-MS/MS gradient, which was isocratic 10% water and 90% methanol based (Line 151). Carotenoids like those studied should not be soluble in these solvents!

I think the authors should either shorten their manuscript or extend it to provide also mass data for the other compounds to better characterize the profile. Currently, I cannot recommend the manuscript for publication unless its majorly revised.

Minors:

Highlights do not fulfill the requirements of the journal.

Language use could be optimized.

-**Reviewer 2**

-

I am very impressed with the presented manuscript. All technical tools used by the authors are very advanced. The research methods used do not raise any objections. The presentation of the results is very good. The authors also satisfactorily displayed the results and discussed them in comparison with world literature. On this basis, I believe that the article is very valuable and should be published in the form presented.

-**Reviewer 3**

-

The manuscript written by Heriyanto et al. introduced the composition of carotenoid pigments from the fruit of the Pandanus plant, *Pandanus conoideus* Lam., indigenous to Papua Islands. This study well evaluated the main components of the red fruit carotenoids to be κ-cyclic carotenoids and their intermediates in the proposed biosynthetic pathway, and discussed the hypothetical biosynthetic route for the κ-cyclic carotenoids in red fruit. It is an interesting topic. This manuscript is well written and easy to follow. Therefore, I think the manuscript can be accepted for publication after a major revision. Specific comments are listed as below.

Introduction

The introduction explains the objectives. However, it is not written clearly. It could be improved.

Lines 51-56: Confusing sentence. Please re-write.

Material and Methods

How many times of experiments were repeated in the article? Please add it.

The detailed information on experimental design and statistical analysis should be described in the paper.

Line 182: 2.7. The spectroscopic data for structural identification. Is it possible to cite references to simplify this part?

Results:

-

Discussion:

-

References:

Do not have consecutive page numbers, and each line starts on a different page, but corrections are made in the order they appear. Please pay attention to all the reference formats, they should be unified.

Line 8:

Line 11:

Line 13:

Line 4:

Line 11:

Line 22:

They should be written in unified formats.

-**Reviewer 4**

-

The manuscript is very interesting and should be published in the Journal of Food Composition and Analysis after review. The main positive point is the chemical characterization of the major carotenoids of red fruit, a native *Pandanus* species of Papua. These carotenoids have special *k*-end group and are described in a group of limited species. However, I suggest that the biosynthetic route based on the absence of minor carotenoid precursors should be better presented and discussed.

1- Introduction. Line 21. Please, insert "monocot" after "*Pandanus conoideus* Lam."

2- Material and Methods Line 78. ..." extraction of the residue was continued with the addition of 1 mL extraction solvente...".

How many times? Was there a complete lack of color in the extraction residue? Please, describe whether the extraction process performed was sufficient to fully extract minor carotenoids, such as anteraxanthin. The amount of sample used in the extraction (0.2 g) was also very small. I have doubts as to whether this amount of sample would be sufficient to completely extract all the minor carotenoids. The extraction performed need to be better described.

3- Line 80. "...saponification of the extract was carried out according to the method of (Kurniawan et al., 2019)."

What was used in the saponification? 10 or 20% KOH? Describe whether the saponification process performed was sufficient to allow complete release of minor esterified carotenoids, such as beta-cryptoxanthin and antheraxanthin. Total saponification of *Capsicum* carotenoids generally requires a more concentrated KOH solution.

4- Results and discussion. Line 357. "...3-hydroxy-beta-end group and others were minor species with the relative concentration of only 0.69%"

This value (0,69%) is not clear. Please, see the table and describe the 3-hydroxy-beta-end compounds (capsanthin + zea + beta-cripto + antheraxanthin = ??? Capsanthin has one β-end and one κ-end group. It is not clear).

5- Line 363. "...the main 5 purified carotenoids in red fruit were unequivocally identified as **5,6-diepicapsokarpoxanthin**,capsorubin, capsanthin, cryptocapsin, and 13-*cis* isomer of capsorubin".

It is not clear. The table 1 shows 0.7% 5,6 diepicapsokarpoxanthin and 3% capsanthin 3,6-epoxide. Please, see the table and rewriter (or explain better) this sentence.

6- Line 398-400. "...we were unable to detect any precursors such as antheraxanthin, and violaxanthin as well as zeaxanthin, although they were detected in the red chili pepper under the same analytical conditions."

Were the carotenoids of red fruit exaustively extracted and saponified? Please, see the comments in "Material and Methods"..

7- Line 402-404. " Collectively, it is likely to suggest the separate biosynthesis route is present in the red fruit κ-cyclic carotenoids which are solely produced from β-cryptoxanthin as a primary precursor".

Red fruit is monocot, such as lily tiger. I think that both species should present the same route. Please, see the item above and comments in the "Material and methods".

-**Reviewer 5**

-

Manuscript number: JFCA_2020_660

Title :Carotenoids from Buah Merah (Pandanus conoideus), an Indigenous Red Fruit of Papua: Compositional Similarity to the Fruit of Red Chili Pepper (Capsicum annuum)

This paper examined the composition of carotenoid pigments from the fruit of the Pandanus plant, and discussed a unique biosynthetic route of carotenoids with the κ-end group in red fruit. While the topic is interesting, issues with approach and presentation make this submission inappropriate for publication.

Minor comments:

1.Highlights: The first two highlights may be deleted and replaced by the detection method in this paper.

2.Abstract: the detection methods may be added.

3.No quantitative analysis of carotenoids detected in this paper.

4.Conclusion need to be supplemented.

5.Please indicate the practical application of this analysis about the composition of carotenoid pigments from the fruit of the Pandanus plant.

-**Reviewer 6**

- No comments

REQUIREMENTS CONCERNING ALL JFCA SUBMISSIONS:

Please copy and paste this entire list into the Detailed Reponse to Editor and respond to each point, and specify line numbers (in revised document) of all edits

1. PAPERS MUST BE REVISED USING TRACKED CHANGES, CLEARLY SHOWING DELETED AND MODIFIED TEXT ALONG WITH ANY NEW TEXT THAT HAS BEEN ADDED. The editors/reviewers must be able to see immediately what has been deleted as well as what has been added.

2. PROOFREADING/SPELLING: Before resubmitting your manuscript, proofread it and run it through a spell checker.

3. TITLE PAGE: The paper must include a title page (usually this is page 1 of your article; it should not be a separate file) indicating the type of paper (Original Research Article, Commentary, Short Communication, Study Review, etc.); the full title; authors names in the order agreed upon by all authors (any changes after submission must be accompanied by a statement signed by all authors); correct affiliation information for each author (does not have to include an email for each author); and the contact information for the Corresponding Author (at least a valid email address). Disclaimers and acknowledgments may be included on the title page, or be placed between the Conclusion and References at the end of the manuscript. (IMPORTANT: ELSEVIER POLICY CONCERNING CHANGES TO AUTHORSHIP AFTER INITIAL SUBMISSION:Any addition, deletion or rearrangement of author names in the authorship list should be made only before the manuscript has been accepted and only if approved by the journal Editor. To request such a change, the Editor must receive the following from the corresponding author: (a) the reason the name should be added or removed, or the author names rearranged, and (b) written confirmation (this can be a signed and scanned statement included with the Cover Letter when the revision is submitted) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed.)

4. HIGHLIGHTS: Highlights are a short collection of bullet points (not a paragraph; and not a repetition of your abstract) that convey the core findings and provide readers with a quick textual overview of the article. These 3-5 bullet points describe the essence of the research (e.g. results or conclusions) and highlight what is distinctive about it. Please see Elsevier instructions on how to write the highlights (http://www.elsevier.com /highlights)

NOTE THE LENGTH RESTRICTIONS ON HIGHLIGHTS: Each highlight must be no more than 85 characters, including spaces.

5. ABSTRACT: The abstract, consisting of approximately 200 words or fewer and in ONE paragraph, must precede the text in the “Manuscript” file, and it must briefly summarize major findings, INCLUDE ACTUAL DATA, and give conclusions. Statements such as “Results are discussed” should not be used. Do not cite references or refer to tables or figures in the abstract.

6. KEYWORDS: 8-10 keywords, INCLUDING THE TERMS “Food analysis” and “Food composition”, must be listed immediately after the abstract. Well-chosen keywords will help other researchers to find and eventually cite your paper. Latin names (botanical, zoological) must be included at least in the keywords where appropriate to ensure proper identification (in general they should also be included in the Sampling section of the paper as well).

7. REFERENCES: Reference citations must be accurate; be sure to check that the spelling of the authors’ names in text citations of references corresponds to every reference listed at the end of the paper. Check for accuracy of dates, article titles, and source titles. “In press” will be queried should your paper be accepted.

8. TABLE AND FIGURE FILES: All tables must be collected together into one file (e.g. Word, Excel) and any figures that are submitted as word-processing files (.doc, .txt, .rtf) must be collected together as another file. High-resolution figures produced in image-software formats (JPG, TIF, EPS, etc.) may be presented as separate files, e.g. 1 figure per file. PLEASE CHECK THAT THERE IS A CAPTION FOR EACH TABLE AND FIGURE.

IMPORTANT NOTE CONCERNING ARTWORK/FIGURES: For basic details concerning digital artwork (size, format) please refer to the online JFCA Guide for Authors, http://www.elsevier.com/journals/journal-of-food-composition-and-analysis/0889-1575/guide-for-authors#57200, under "Artwork"; for more detailed information (e.g. meaning of "low/high resolution", image formats, etc.) please visit: http://www.elsevier.com/author-schemas/artwork-and-media-instructions

9. ABBREVIATIONS: Any abbreviations used in the Abstract must be spelled out at first use within the Abstract. Any abbreviations used in text must be spelled out at first use within the text (independently of use in the Abstract). Any abbreviations used within Tables or Figures must be defined within the same Table or Figure; in other words, each table or figure contains definitions of abbreviations, even if they are used in more than one table.

10. TECHNICAL DETAILS TO BE CHECKED IN YOUR REVISED PAPER

* The limit of detection (LOD) must be given whenever "not detected" values are being reported; the LOD must be presented in the same units as those of sample data; and the LOD must be specified wherever "not detected" was cited in a data table (e.g. in a footnote).

* If a CERTIFIED REFERENCE MATERIAL is analysed, actual results must be reported. In addition, the name/matrix of the material must be specified, and the supplier and the certified range for each nutrient measured must be given.

* SUPPLIER DETAILS: the purity and supplier for all standards, the supplier and grade of all chemical reagents, the manufacturer of all equipment, and the source of any statistical or other software programs must be specified (generally in the Methods section). Supplier and manufacturer location (city, state where applicable, country) must be included within parentheses after the first citation of a given supplier/manufacturer. Examples: "A Shimadzu ICPS-1000 (Shimadzu Corporation, Tokyo, Japan)..."; "a fluoride-selective electrode Cole-Parmer 27502-19 (Cole-Parmer, Vernon Hills, IL, USA)..."; "software Matlab® 2009b"... (http://fr.mathworks.com/support/sysreq/release2009b/).

* STATISTICAL SIGNIFICANCE must be shown for all "differences" found in data.

* SIGNIFICANT DIGITS in data reporting: Concentration values must be written to NOT MORE THAN three significant figures (data in text, tables and figures). Examples: the numbers 123, 12.3, 1.23, 0.123, and 0.0123 all have three significant digits.

For more detailed explanations on conventions used for food composition data, see Greenfield, H. and D.A.T. Southgate, 2003; Food composition data: Production, Management and Use; Rome, FAO; see especially Chapter 9 and Table 9.1. (http://www.fao.org/docrep/008/y4705e/y4705e00.htm)

* n, and statistical parameters must be clearly defined in Tables and Figures, and appropriate error bars must be shown in the Figures.

* The International System of Units (SI, Système International d'Unités), or the SI-derived system, is to be used in reporting units of measurement. Decimal points (not commas) must be used. If other units are mentioned (quarts, pounds, ounces, miles, cups, teaspoons, etc.), please give their equivalent in SI. Energy is to be expressed as kJ or MJ (and not as calories; equivalent kcal or Mcal may be given in parentheses). The Celsius scale (°C) should be used for temperature. Please also note: L, mL, µL (not

l, ml, µl). More information about SI units and abbreviations can be found here: http://www.bipm.org/en/measurement-units/

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Manuscript Number: JFCA_2020_660R1

Carotenoids from Buah Merah (Pandanus conoideus), an Indigenous Red Fruit of Papua: Compositional Similarity to the Fruit of Red Chili Pepper (Capsicum annuum)

Dear Dr Brotosudarmo,

Thank you for submitting your manuscript to Journal of Food Composition and Analysis.

I have completed my evaluation of your manuscript. The reviewers recommend reconsideration of your manuscript following minor revision and modification. I invite you to resubmit your manuscript after addressing the comments below. Please resubmit your revised manuscript by Oct 22, 2020.

When revising your manuscript, please consider all issues mentioned in the reviewers' comments carefully: please outline every change made in response to their comments and provide suitable rebuttals for any comments not addressed. Please note that your revised submission may need to be re-reviewed.

To submit your revised manuscript, please log in as an author at https://www.editorialmanager.com/jfca/, and navigate to the "Submissions Needing Revision" folder under the Author Main Menu.

Journal of Food Composition and Analysis values your contribution and I look forward to receiving your revised manuscript.

Kind regards,

Katherine Phillips

Handling Editor

Journal of Food Composition and Analysis

Editor and Reviewer comments:

Reviewer #1: In the first review, I criticized that methanol as HPLC injection solvent might not have quantitatively dissolved the carotenoids that had been extracted with ethanol/hexane. The authors agree with this view, stating that indeed acetone as injection solvent led to higher HPLC peaks for apolar carotenoids like beta-carotene. However, this higher intensity was achieved at the expense of peak separation.

That's a very common dilemma, many scientists face this issue. Thorough method

development commonly allows to find solutions. In extreme cases, this might even require two LC runs for one sample. Commonly, making up nitrogen-dried extracts with mixtures of methanol and MTBE provides sufficient polarity range and good chromatography results. Now, in this case, given the fact that the authors present the first detailed report about the carotenoids in this fruit, the paper still contains valuable information and, in my view, the publication process can be continued in the journal, but only if they briefly but clearly address this potential limitation in the paper itself - not only in the response letter. This will help future readers, if they find deviating results (such as additional apolar pigments or at least higher levels of those reported herein!). It will also help readers that are trying to transfer your method to something else. Finally, it will avoid discussions in
future reviews, where other scientists might argue with the fact that they had used a previously published (but still limited!) method to justify suboptimal work-up procedures.

My other major concerns have been adequately addressed. I particularly welcome the inclusion of analytical data obtained with an analytical C30 column, more detailed MS data now and much more careful expressions when talking about the biosynthesis of the analyzed compounds. Except for the sub-optimal extraction work-up procedure, the paper is very well done and merits publication after thorough improvement of some minor issues listed below.

Minor suggestions for edits:

I think the highlights still do not comply with the journal requirements (85 characters rule!).

In Table 1, you may want to replace "Relative" with "Relative Abundance" or similar to clarify that its about quantity without having to look into the
Table header. Just a suggestion.

Peak 40, lutein, please check again if you find the in-source fragment [M+H-H2O] in the extract. This commonly is the major ion for lutein in MS1. Was this in-source fragment absent in Buah Merah extracts, too?

You may want to find a better photograph for Figure 1a, because the current one doesn't allow to really see the single fruits yet (at least on my screen). Maybe, you also just need to play a little bit with contrast/brightness.

In the supplementary materials, there is a Table on page 12 that has no Table numbering. And I think it is unclear what the data in the Table actually shows. Please improve and clarify.

On page 10, there is also a Table without Table numbering, please correct.

Please also improve Figure numbering in the Supplementary. There are Figs. 7B and C, but the label for S7A is missing. The captions for Figs. S7B and C must be improved in my view. I guess some information from S7A is also relevant for these Figures?

Reviewer #3: The results of my comments are acceptable. The manuscript can be published in the Journal of food composition and analysis.

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Data in Brief (optional):

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