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Short communication

Determination of carotenoids in tomato juice by liquid chromatography

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Abstract

A high-performance liquid chromatography method was developed to determine the various carotenoids in tomato juice. A C_{30} column and a mobile phase of acetonitrile–1-butanol (7:3, v/v) (A) and methylene chloride (B) with the following gradient elution were used: 99% A and 1% B intitally, increased to 4% B in 20 min, 10% B in 50 min and returned to 1% B in 55 min. Sixteen carotenoids, including all-*trans*-lutein, all-*trans*-β-carotene, all-*trans*-lycopene and their 13 *cis* isomers were identified and resolved within 52 min with flow-rate at 2.0 ml/min and detection at 476 nm. Of the various extraction solvent systems, the best extraction efficiency of carotenoids in tomato juice was achieved by employing ethanol–hexane (4:3, v/v). Lycopene was found to be present in largest amount in tomato juice, followed by β-carotene and lutein.

Keywords: Fruit juices; Food analysis; Carotenoids

1. Introduction

Epidemiological studies have shown that the increased consumption of tomato and tomato-based products may reduce the risk of a certain type of cancers such as prostate and stomach cancer [1]. One of the major phytochemicals in tomato products contributing to the anti-carcinogenic function has been attributed to lycopene [2,3]. In addition to lycopene, both lutein and β-carotene are also present in tomato in a much smaller amount [4]. The application of lutein and β-carotene in the treatment of chronic diseases such as age-related macular degeneration and skin cancer have been well documented [5].

Lycopene, an acyclic carotenoid containing 11 conjugated double bonds, is naturally present in trans form in raw tomato [6]. Because of presence of long-chain conjugated double bonds, lycopene has been reported to possess antioxidative activity and is superior to lutein or β-carotene [7]. In addition, lycopene may exhibit other physiological activities such as suppression of proliferation of human cancer cells [8]. However, all-*trans*-lycopene may be converted to its cis configuration during food processing [9]. Several reports have demonstrated that the *cis* isomers of lycopene could be absorbed into body more easily and played a more important role in biological function than all-*trans*-lycopene [10,11].

The consumption of tomato juice in Taiwan has increased rapidly in recent years. However, the

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amount and variety of *cis* carotenoids remain unclear in processed tomato juice due to lack of an appropriate high-performance liquid chromatography (HPLC) technique [12], though a gradient mobile phase of acetone and water was developed to determine the various carotenoids in tomato peel [12]. Also, the effect of various solvent systems on the extraction efficiency of carotenoids in tomato juice need to be compared. The objectives of this study were to develop a suitable extraction and separation method for determination of carotenoids in tomato juice by HPLC.

2. Experimental

2.1. Materials

Fresh tomatoes (*Tan-Tai Lan T93*) were purchased from a local farm in Taichung county. All-*trans*-lutein and all-*trans*-β-carotene standards with purities greater than 95% were obtained from Sigma (St. Louis, MO, USA). All-*trans*-lycopene standard was from Extrasynthese (France). The HPLC-grade solvents, including ethanol, acetone, tetrahydrofuran, *n*-hexane, methanol, acetonitrile, ethyl acetate, methylene chloride and methyl *tert*.-butyl ether (MTBE) were from Mallinckrodt (Paris, KY, USA). 1-Butanol and sodium chloride were from Riedel-de Häen (Barcelona, Spain). The deionized water was made using a purified-water system (Millipore, Bedford, MA, USA). Magnesium carbonate was from J.T. Baker (Pillipsburg, NJ, USA). A C_{30} column (250×4.6 mm I.D, 5 μm particle) was from YMC (Tokyo, Japan).

Tomato juice was processed using a HTST (High-Temperature-Short-Time) system and was obtained from a pilot plant located in the Hsin-Chu county of Taiwan.

2.2. Instrumentation

The HPLC system is composed of a Jasco MD-915 photodiode-array detector, a Rheodyne model 7161 injector (Rheodyne, CA, USA) and a Sanwa Tsusho DP-4010 degasser (Sanwa Tsusho, Tokyo, Japan). The homogenizer (model PT-MR 3000) was from Kinematica (Switzerland). The rotary evaporator (model N-1) was from Eyela (Tokyo, Japan).

2.3. Extraction of carotenoids from tomato juice

Five solvent systems were used for comparison of extraction efficiency: (1) ethanol–hexane (4:3, v/v), (2) acetone–hexane (3:5, v/v), (3) ethanol–acetone–hexane (2:1:3, v/v/v), (4) ethyl acetate–hexane (1:1, v/v) and (5) ethyl acetate (100%). These solvent systems were selected based on several reports of previous studies [13–15]. Initially a 8 g sample of tomato juice was placed in a 60 ml vial and mixed with 0.2 g magnesium carbonate and 40 ml extraction solvent as described above. The mixture was shaken in a shaker at 140 rev./min for 30 min. The upper phase was collected and poured into a 500-ml flask. The lower phase was extracted again with the same solvent (32 ml) and shaken for 30 min. The upper phase was also collected and poured into the same flask. The lower phase was repeatedly extracted with 15 ml hexane and shaken for 20 min, followed by 5 ml hexane and homogenized at 12 000 rev./min for 5 min. After filtration through a Whatman No. 1 filter paper, the filtrates were pooled and poured into the same flask. Distilled water (150 ml) and 100 ml 10% aq.NaCl solution were added for partition, and the supernatant was collected. The lower phase was again extracted with 20 ml hexane. The filtrates were combined and evaporated to dryness in a flask under vacuum. The residue was dissolved in 1 ml methylene chloride and transferred to a vial. The solution was filtered through a 0.2-μm membrane filter and 20 μl was injected for HPLC analysis.

2.4. Determination of recovery

For recovery test, a concentration of 30 μg/ml all-*trans*-lutein and 100 μg/ml all-*trans*-β-carotene were added to 8 g tomato juice separately for extration of carotenoids. For all-*trans*-lycopene, a concentration of 250 μg/ml was added to 2 g tomato juice instead of 8 g for extraction. This is because that a large concentration was found for all-*trans*-lycopene in tomato juice, and the amount of all-*trans*-lycopene standard incorporated would be much greater if 8 g tomato juice sample was used. The

recovery of each all-*trans* form of carotenoids was obtained by dividing the calculated concentration by the added concentration. The recoveries of all-*trans*-lutein, all-*trans*-β-carotene and all-*trans*-lycopene were found to be 76, 84 and 94%, respectively. Because of absence of commercial standards of *cis* form of carotenoids, the recoveries of *cis* isomers were assessed to be equivalent to those of all-*trans* form of carotenoid standards.

2.5. HPLC separation of carotenoids in tomato juice

Various binary and ternary solvent systems in isocratic or gradient mode were compared with respect to the separation efficiency of carotenoids in tomato juices. These solvent systems were selected and modified based on several previous studies by Lee and Chen [6] and Emenhiser et al. [16]. A proper solvent strength was controlled for each mobile phase by calculating the polarity index. The most appropriate solvent system was found to be composed of 1-butanol–acetonitrile (30:70, v/v) (A) and methylene chloride (B) with the following gradient elution: 99% A and 1% B initially, increased to 4% B in 20 min, 10% B in 50 min and returned to 1% B in 55 min. The flow-rate was 2.0 ml/min with detection at 476 nm and sensitivity at 0.005 AUFS. The separation efficiency was evaluated on the basis of retention factor (κ).

2.6. Identification and quantification of carotenoids in tomato juice

The identification of all-*trans* carotenoids was carried out by comparing the retention times and absorption spectra with reference standards, as well as co-chromatography with added standards. In addition, the *cis* isomers of carotenoids were identified based on absorption spectrum characteristics and *Q* ratios as described in the literature [6,20–23].

Because of an absence of suitable internal standard, six concentrations of 1, 2, 4, 8, 10 and 20 μg/ml were used to prepare the standard curve of all-*trans*-lutein. Likewise, six concentrations of 3, 6, 18, 36, 48 and 60 μg/ml were prepared for the standard curve of all-*trans*-β-carotene. For all-*trans*-lycopene, two standard curves were prepared since a great difference in concentration was found between all-*trans* and *cis* form of lycopene in tomato juice. Six concentrations, 7, 70, 140, 280, 560 and 700 μg/ml were prepared for one curve, whereas 2, 4, 8, 10, 20 and 40 μg/ml were prepared for the other. Each *cis* isomer of carotenoids was quantified based on the standard curve of all-*trans* carotenoids because of similarity in extinction coefficient [9,17]. Duplicate analyses were performed and the mean value was determined. The regression equation and correlation coefficient (r^2) were obtained using a Microsoft Excel 2000 software. A high correlation coefficient ($r^2 > 0.98$) was achieved for all-*trans*-β-carotene ($y = 0.0141x + 0.8907$), all-*trans*-lutein ($y = 0.0083x - 0.0807$) and all-*trans*-lycopene ($y = 0.0068x + 19.833$ for high concentration and $y = 0.0069x + 1.0442$ for low concentration). The data were subjected to analysis of variance and Duncan's multiple range test using SAS [18].

2.7. Determination of limits of detection and quantification

Both the limit of detection (LOD) and limit of quantification (LOQ) were determined using a method described by International Conference on Harmonization [19]. Due to difference in detector response, three concentrations (1, 2 and 4 μg/ml) were prepared for all-*trans*-lutein and all-*trans*-lycopene separately, while 3, 6 and 18 μg/ml were for all-*trans*-β-carotene. Both LODs and LOQs were calculated based on a formula in a previous study [6]. The LODs for lutein, β-carotene and lycopene were 0.64, 0.65 and 0.09 μg/g, respectively, while the LOQs were 1.94, 1.98 and 0.27 μg/g.

3. Results and discussion

3.1. HPLC analysis of carotenoids in tomato juice

After various studies, a gradient mobile phase as described in the method section was developed, which was able to resolve 16 carotenoids in tomato juice (Fig. 1). Table 1 shows the chromatographic data for all-*trans* and *cis* forms of lutein, β-carotene and lycopene in tomato juice. With the exception of all-*trans*-lycopene, the peak purities of all 15 carot-

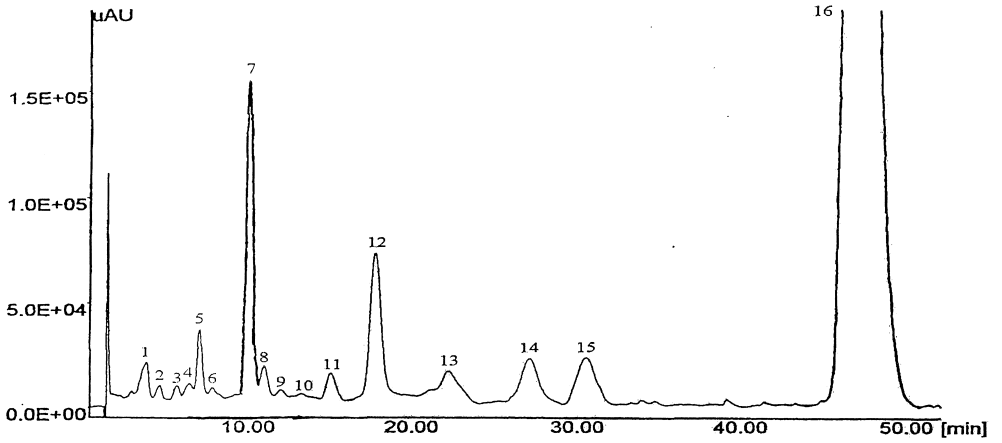

Fig. 1. HPLC chromatogram of carotenoids in processed tomato juice by employing a gradient system of 1-butanol, acetonitrile and methylene chloride. Chromatographic conditions described in text. See Table 1 for peak idenification.

Table 1
Tentative identification and chromatographic data for all-trans and cis forms of carotenoids in processed tomato juice

Peak no.	Compound	Retention time (min)	κ[a]		λ (nm) (in-line)[b]				λ (nm) reported			Q-ratio found	Q-ratio reported
1	all-*trans*-lutein	4.23	1.26		422	446	476		426	443	474[d]	–	–
2	9-*cis*-lutein	5.03	1.69	356	428	446	476		420	442	467[d]	0.19	$0.12(8.6^{-1})$[d]
3	13-*cis*-lutein	6.08	2.26	374	434	458	488		419	439	465[d]	0.34	$0.38(2.6^{-1})$[d]
4	di-*cis*-β-carotene	6.84	2.66	350	404	422	458		(413)	437	(458)[d]	0.68	–
5	15-*cis*-β-carotene	7.45	2.99	344	(422)	446	476		(421)	443	470[e]	0.37	$0.43(2.3^{-1})$[e]
6	9-*cis*-β-carotene	8.21	3.40	344		452	476		(420)	442	469[f]	0.10	$0.12(8.2^{-1})$[f]
7	all-*trans*-β-carotene	10.39	4.56			458	482		(417)	453	477	–	–
8	*cis*-β-carotene	11.30	5.05		422	452	476			–[c]		–	–
9	13-*cis*-β-carotene	12.31	5.60	344	(422)	458	476		(419)	442	465[g]	0.20	$0.35(2.8^{-1})$[g]
10	9,13′-di-*cis*-lycopene	13.61	6.29	368		458	488	350		458[h]		0.20	–[i]
11	15-*cis*-lycopene	15.44	7.27	362	446	470	500	362	446	470	506[h]	0.61	0.75[h]
12	13-*cis*-lycopene	18.15	8.72	362	440	470	508	362	446	470	500[h]	0.55	0.55[h]
13	9,13-di-*cis*-lycopene	22.53	11.07	344	440	464	494		434	464	494[h]	0.10	–[i]
14	9-*cis*-lycopene	27.47	13.71	362	446	470	500	362	446	470	500[h]	0.12	0.12[h]
15	5-*cis*-lycopene	30.92	15.56	344	446	476	506	368	452	476	506[h]	0.05	0.06[h]
16	all-*trans* lycopene	47.37	24.37		452	476	506		452	476	506[h]	–	–

[a] κ, retention factor.
[b] A gradient mobile phase of 1-butanol–acetonitrile (30:70, v/v) and methylene chloride was used.
[c] "–" Data not available.
[d] A mobile phase of methanol–methylene chloride (99:1, v/v) was used by Chen et al. [23].
[e] A mobile phase of acetonitrile–methanol (90:10, v/v) was used by Chen and Chen [22].
[f] A mobile phase of acetonitrile–methanol–methylene chloride was used by Saleh and Tan [21].
[g] A mobile phase of acetone–hexane (3:97, v/v) was used by Tsukida et al. [20].
[h] A mobile phase of 1-butanol–acetonitrile–methylene chloride (30:70:10, v/v/v) was used by Lee and Chen [6].
[i] Based on a reference by Olaser and Albert [12].

Table 2
Concentrations (μg/g)[a] of lutein, β-carotene and lycopene in tomato juice after extraction with various solvent systems

Treatment	Lutein				β-carotene						Lycopene							
	All-*trans*	9-*cis*	13-*cis*	Total	15-*cis*	9-*cis*	All-*trans*	*cis*[f]	13-*cis*	Total	Di-*cis*(9,13′)	15-*cis*	13-*cis*	Di-*cis*(9,13)	9-*cis*	5-*cis*	All-*trans*	Total
A[b]	0.37^{A}	0.14^{A}	0.13^{B}	0.64^{A}	1.03^{A}	0.33^{A}	4.05^{A}	0.37^{A}	0.25^{A}	6.03^{A}	0.18^{AB}	0.51^{A}	2.57^{A}	1.03^{A}	0.80^{A}	2.70^{A}	113.87^{A}	121.66^{A}
B[c]	0.37^{A}	0.13^{AB}	0.19^{A}	0.69^{A}	0.82^{C}	0.28^{B}	3.43^{C}	0.34^{AB}	0.23^{AB}	5.1^{C}	0.17^{BC}	0.28^{C}	0.99^{C}	0.76^{C}	0.66^{C}	1.71^{D}	67.17^{E}	71.74^{E}
C[d]	0.32^{B}	0.14^{A}	0.13^{B}	0.59^{AB}	0.87^{B}	0.32^{A}	3.79^{B}	0.36^{A}	0.25^{A}	5.59^{B}	0.18^{A}	0.37^{B}	1.59^{B}	0.88^{B}	0.72^{B}	2.19^{B}	90.20^{B}	96.13^{B}
D[e]	0.26^{C}	0.10^{B}	0.08^{C}	0.44^{C}	0.83^{C}	0.26^{C}	3.21^{D}	0.32^{B}	0.21^{B}	4.83^{D}	0.16^{C}	0.35^{B}	1.42^{BC}	0.79^{C}	0.65^{C}	1.89^{C}	72.62^{D}	77.88^{D}
E[f]	0.30^{BC}	0.10^{B}	0.12^{B}	0.52^{BC}	0.81^{C}	0.26^{C}	3.27^{CD}	0.34^{AB}	0.22^{AB}	4.9^{CD}	0.17^{C}	0.29^{C}	0.96^{C}	0.79^{C}	0.69^{C}	1.88^{C}	77.84^{C}	82.62^{C}

[a] Mean of duplicate analyses. $^{A-E}$Symbols bearing different letters in the same column are significantly different ($P<0.05$).
[b] **A** = ethanol–hexane (4:3, v/v).
[c] **B** = acetone–hexane (3:5, v/v).
[d] **C** = ethanol–acetone–hexane (2:1:3, v/v/v).
[e] **D** = ethyl acetate–hexane (1:1, v/v).
[e] **E** = ethyl acetate (100%).
[f] Unidentified *cis*-β-carotene.

enoids were higher than 90%. The κ values of all 16 carotenoids were between 1.26 and 24.37, indicating that a proper solvent strength of mobile phase was controlled. The κ values observed in this study were a bit higher, mainly because a large variety of carotenoid isomers were present in tomato juice, and a great hydrophobic interaction could occur between C_{30} stationary phase and carotenoid isomers, which in turn resulted in a prolonged retention time [17]. Peaks 1, 7 and 16 were positively identified as all-*trans*-lutein, al-*trans*-β-carotene and all-*trans*-lycopene, respectively, based on the criteria described in the Method section. Peaks 2 and 3 were tentatively identified as 9-*cis*-lutein and 13-*cis*-lutein, respectively, because a low and high intensity of *cis* peak occurred for the former and the latter. In addition, a hypsochromic shift of 8 nm was found for 13-*cis*-lutein. Peaks 4, 5, 6, 8 and 9 were tentatively identified as di-*cis*-, 15-*cis*-, 9-*cis*-, *cis*- and 13-*cis*-β-carotene, respectively, because a hypsochromic shift of 36, 12, 6, 6 and 6 nm occurred and the *Q*-ratios were similar to those reported in the literature [20–23]. No cis position was assigned to peaks 4 and 8 since no *Q*-ratio in the literature could be compared. However, a large hypsochromic shift and exclusion of the sterically-hindered isomers such as 7-*cis*- and 11-*cis*-β-carotene may indicate the presence of di-*cis*-β-carotene as reported by Chen et al. [24]. Peaks 10, 11, 12, 13, 14 and 15 were tentatively identified as 9,13′-di-*cis*-, 15-*cis*-, 13-*cis*-, 9,13-di-*cis*-, 9-*cis*- and 5-*cis*-lycopene, respectively, based on a hypsochromic shift of 16, 6, 6, 12, 6 and 0 nm and the Q-ratios [6,25], as well as a reference by Oleser and Albert [12].

3.2. Comparison of extraction efficiency

Table 2 shows the concentrations of lutein, β-carotene and lycopene in tomato juice after extraction with various solvent systems. Lycopene was found to be present in largest concentration, followed by β-carotene and lutein. Of the various solvent systems, ethanol–hexane (4:3, v/v) resulted in the highest yield of lycopene, followed by ethanol–acetone–hexane (2:1:3, v/v/v), ethyl acetate (100%), ethyl acetate–hexane (1:1, v/v) and acetone–hexane (3:5, v/v). The same trend also applied to lutein and β-carotene. Compared to all-*trans* carotenoids, the *cis* carotenoids were present at a much lower level. As most carotenoids are present naturally in *trans* form in plants, the formation of *cis* isomers is probably due to processing. Taungbodnitham et al. [26] compared different combinations of solvent systems for extraction efficiency of carotenoids in fruits and vegetables and concluded that both acetone–hexane (4:6, v/v) and ethanol–hexane (4:3, v/v) resulted in a better extraction yield of lycopene. However, no difference was found for β-carotene. In our study the highest yield of lycopene, lutein and β-carotene in tomato juice could be simultaneously obtained by using ethanol–hexane (4:3, v/v) as extraction solvent. The amounts of all-*trans* and *cis* forms of carotenoids in tomato juice could be affected by many factors such as variety and maturity of tomatoes as well as processing condition. In addition, the extraction and separation techniques used by the other authors may also cause this difference. Shi and Maguer [27] reported that the content of lycopene ranged 5.8–9.0 mg/100 g in tomato juice produced in Israel and 9.70–11.84 mg/100 g in Brasil on a wet basis. These values were a bit lower than that shown in our study, in which the lycopene content (all-*trans* plus *cis* form) in tomato juice was 12.17 mg/100 g.

In conclusion, the most appropriate HPLC method for separation of 16 carotenoids in tomato juice was accomplished by employing a C_{30} column with a gradient mobile phase of acetonitrile–1-butanol (7:3, v/v) and methylene chloride. A solvent system of ethanol–hexane (4:3, v/v) was found to result in the highest yield of carotenoids extracted from tomato juice.

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