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Comparative study on the carotenoid composition of the peel and the pulp of different citrus species

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Abstract

The carotenoid compositions of the peel and the pulp of various citrus fruits were compared with HPLC methods using C18 and C30 columns. The extracts usually contain β-cryptoxanthin and lutein in considerable amounts and in all species except lime, the red apocarotenoid β-citraurin as well. In case of lime and mandarin the carotenoid compositions of peel and pulp show a good coincidence while in orange, clementine, grapefruit, lemon and kumquat there are a lot more differences. Lime extracts contain practically only two carotenoids: β-carotene and lutein. The carotenoid components of the saponified extracts of kumquat were separated on calcium carbonate columns and were investigated in detail. The components were identified with HPLC–DAD and HPLC–MS.

Keywords: Carotenoids; Citrus fruits; Kumquat; HPLC–MS

Industrial relevance: Citrus fruits are important starting materials for juice production. Their carotenoid fingerprint shows differences not only in different species but the proportion of certain pigments can be different in the same fruit according to where the plants were grown and how they were processed. Comparison of the carotenoid content of different fruit products (e.g. juices) can give us useful hints about the quality of the product and about the amount of these important natural antioxidants.

1. Introduction

Dietary carotenoid antioxidants from fruits and vegetables have long been known to play an important role in human health. For their implication in cancer prevention there is a growing interest in the antioxidant composition of various fruits and vegetables. Citrus fruits contain a considerable amount of carotenoids and form part of the daily nutrition of the people. That is why in the recent years a lot of articles have focused on the investigation of these fruits. However, these studies focused only on the major carotenoid components and were restricted to various orange species and orange juices (Curl & Bailey, 1956; Dhuique-Mayer, Caris-Veytrat, Ollitrault, Curk, & Amiot, 2005; Gil-Izquierdo, Gil, & Ferreres, 2002; Lee & Castle, 2001; Meléndez-Martínez, Britton, Vicario, & Heredía, 2005; Mouly, Gaydou, Lapierre, & Corsetti, 1999; Schlatterer, & Breithaupt, 2005).

Our present paper reports on the HPLC investigation of the carotenoid composition of fresh citrus fruits (orange (*Citrus sinensis*), mandarin (*Citrus reticulate*), clementine (*Citrus reticulata*), kumquat (*Citrus fortunella*), grapefruit (*Citrus paradise*), lime (*Citrus aurantifolia*) and lemon (*Citrus limon*)) focusing on the up till now not investigated kumquat. The peel and the pulp of the fruits were processed and investigated separately.

2. Materials and methods

2.1. Reagents and standards

During our work, analytical grade chemicals were used. All solvents used in high-performance liquid chromatography (HPLC) were of HPLC grade. Authentic samples (zeaxanthin, lutein, β-cryptoxanthin, β-citraurin, β-carotene, α-carotene, violaxanthin, (9*Z*)-violaxanthin, mutatoxanthin) were taken from our collection.

2.2. Plant materials

The citrus fruits originated from different countries and were purchased in a Tesco and a Spar supermarket in Pécs (Hungary).

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Table 1
Carotenoid composition (%) of different citrus peels

Peak	Carotenoid	Orange	Mandarin	Clementine	Kumquat	Grapefruit	Lemon
1	Unidentified	0.6	0.4	0.2	0.8	1.7	0.5
2	Unidentified	1.4	0.3	0.8	0.8	1.0	0.5
3	Unidentified	2.2	0.6	0.6	0.2	1.1	0.3
4	Unidentified	1.2	0.4	0.7	0.2	5.8	2.5
5	Neochrome	1.6	3.1	5.7	1.5	2.2	4.3
6	Violaxanthin+furanoid	13.6	8.7	1.4	9.8	8.9	0.2
7	Unidentified	–	2.1	0.7	–	–	–
8	β-Citraurin	10.8	13.3	28.0	16.6	9.3	4.0
9	(*Z*)-β-citraurin	0.9	1.0	2.1	1.4	1.5	0.1
10	(*Z*)-β-citraurin	1.0	1.2	2.5	1.0	0.7	0.1
11	Luteoxanthin	2.9	1.6	6.1	3.7	2.1	0.5
12	(9*Z*)-violaxanthin	33.8	18.0	7.9	16.9	6.4	1.6
13	(13*Z*)-violaxanthin	1.4	0.3	0.2	1.7	1.7	0.1
14	Lutein	6.6	5.4	4.1	5.5	7.6	8.3
15	Unidentified	–	–	–	2.1	–	–
16	Unidentified	0.8	0.2	0.6	0.8	1.3	1.3
17	Unidentified	0.8	0.3	1.0	0.8	1.3	1.0
18	Monofuranoid like (λ_{max}=450, 423 nm)	0.9	0.6	0.4	1.1	0.7	1.4
19	Cryptochrome	3.5	2.8	2.8	5.7	4.3	1.4
20	Diepoxide like	0	1.4	1.9	0	0	7.4
21	Unidentified	0.4	0.4	1.6	0.8	1.2	10.9
22	Monoepoxide like (λ_{max}=475, 450 nm)	–	–	–	1.2	1.5	3.1
23	Monofuranoid like (λ_{max}=450, 423 nm)	2.1	4.5	3.7	3.8	2.1	7.9
24	α-Cryptoxanthin	0.3	0.3	0.2	0.2	1.4	1.5
25	β-Cryptoxanthin	3.5	23.4	13.4	11.4	11.3	19.9
26	(*Z*)-β-cryptoxanthin	1.2	3.9	4.0	2.8	1.6	3.6
27	ξ-Carotene	1.2	1.8	0.3	0.9	7.5	7.2

2.3. Pigment extraction

To obtain reliable samples 300–500 g (fresh weight) of fruits were used for extraction. Peel and juice sacs were separated and cut into small pieces. The samples were extracted three times with methanol and then twice with diethylether. The extracts were combined and evaporated and saponified in ether with 30% KOH/MeOH at room temperature. The saponified pigments were stored in benzene solution at − 20 °C, under nitrogen and under exclusion of light until preparation of HPLC samples. General methods including sample taking, extraction, workup and quantitative determination of chlorophyll were described in detail in a previous study of yellow paprika (Matus, Deli, & Szabolcs, 1991). To avoid pigment decomposition, *cis-trans* isomerisation and epoxide–furanoid oxide rearrangement, the isolation of carotenoids was carried out under nitrogen in darkness using methanol for dehydration at low temperatures (4–23 °C).

2.4. High-performance liquid chromatography

HPLC system I for C_{18} column: Dionex pump model 580, Hewlett Packard 1050 detector with HP ChemStation software and Waters-991, photodiode array detector; column 250 × 4.6 mm i.d., Licrospher C_{18} (Merck), 5 μm endcapped; gradient elution with A (12% (v/v) H_2O in methanol), B (methanol), C (30% (v/v) dichloromethane in methanol), i.e. 0–2 min: 100% A; 2–10 min: to 80% A/20% B; 10–18 min: to 50% A/50% B; 18–25 min: to 100% B; 25–27 min: 100% B; 27–34: to 100% C; 34–41 min 100% C (linear steps), flow rate 1.25 ml/min.

HPLC system II for C_{30} column: Dionex pump model P 580 NDG, Dionex 340S diode array detector and Chromeleon software; column: 250 × 4.6 mm i.d. Kovasil C_{30}, 5 μm, endcapped; eluent: A: 10% (v/v) H_2O in methanol, B: *tert*-butyl methyl ether. Gradient program: 0–35 min 8% B–25% B, 35–50 min 25% B–50% B, 50–60 min 50% B–70% B, 60–70 min 70%B, 70–75 min 70% B–8% B (regeneration), flow rate: 1 ml/min.

MS: Finnigan AQA, APCI+polarity without splitting; Crown U: 4 kV, Temp: 400 °C, full scan mode, data acquisition rate: 0.2 scans/s; AQA max voltage: 30 V, mass filter: 10, 20, 30 V.

2.4.1. Identification of the peaks

The total carotenoid contents were determined by UV–VIS methods. Carotenoids were identified on the basis of the same retention times, same spectral characteristics with standards and co-chromatography with authentic samples. The peaks in a chromatogram were identified with the help of authentic carotenoid samples, various chemical tests (Baranyai, Matus, & Szabolcs, 1982; Matus et al., 1991) and the UV–VIS spectra of the individual peaks. Photodiode array measurements of spectral properties for the individual peaks (from 300 to 510 nm) were made at the upslope, apex and downslope of the curves. The matching of the three spectra indicated the degree of peak purity.

2.4.2. Quantification

The chromatograms were evaluated quantitatively by relating the heights of the individual carotenoids to that of canthaxanthin using an internal standard (Matus et al., 1991).