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Technical Report

Characterization of 17 Capsicum varieties by evaluation of their apocarotenoid profile by Nexera™ UC

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Abstract:

An on-line method based on the coupling of supercritical fluid extraction and supercritical fluid chromatography with triple quadrupole mass spectrometry detection (SFE-SFC-MS/MS) for the native apocarotenoids characterization of 17 Capsicum varieties was here reported. 27 compounds were extracted and identified by the developed SFE-SFC-MS/MS methodology, including free apocarotenoids, apocarotenoids monoesters, apocarotenoids diesters and chlorophylls in a very fast, and efficient way.

Keywords: SFE-SFC-MS/MS, Capsicum, Apocarotenoids

1. Introduction

Carotenoid pigments are mainly C40 lipophilic isoprenoids that are commonly present in our daily diet, and have been described as possessing several important functional properties mainly antioxidant activity as well as prevention of cardiovascular diseases, cancer, macular degeneration and in some cases provitamin A activity. Apocarotenoids are carotenoids in which the carbon skeleton has been shortened by removal of fragments from one or both ends. Very important apocarotenoids in the food industry are pigments bixin and crocetin used as food colorants, moreover apocarotenoids are very important in different biological processes in plants, such as regulation of growth and development, and signalling. Apocarotenoids were found in food and in humans, suggesting that these products may play important biological roles and that they may be responsible for the bioactivity. Relatively few studies are available in the literature on the apocarotenoids occurrence in food, mainly based on raw tomato, red grapefruit, and watermelon. The present study reports on the application of the Nexera UC instrument for the apocarotenoids profiling of seventeen different chilli peppers cultivars belonging to Capsicum annuum, Capsicum baccatum and Capsicum chinense species.

2. Experimental

2-1. Samples

Seventeen fresh chilli peppers samples belonging to the genus Capsicum were analyzed, namely: Aji limòn Capsicum baccatum (Sample 1), Erotic Capsicum baccatum (Sample 2), Jimmy Capsicum baccatum (Sample 3), Banana Pepper Capsicum annuum (Sample 4), Cayenna Impala Capsicum annuum (Sample 5), Jalapeño Capsicum annuum (Sample 6), Terenzio Capsicum annuum (Sample 7), Calabrian pepper Capsicum annuum (Sample 8), Scotch Bonnet Capsicum chinense (Sample 9), Habanero Red Savina Capsicum chinense (Sample 10), Habanero Fatalii Capsicum chinense (Sample 11), Habanero Chocolate Capsicum chinense (Sample 12), Naga Morich Capsicum chinense (Sample 13), Naga Yellow Capsicum chinense (Sample 14), Naga Chocolate Capsicum chinense (Sample 15), Trinidad Scorpion Capsicum chinense (Sample 16), Trinidad Scorpion Moruga Capsicum chinense (Sample 17).

2-2. Sample preparation

Fresh chilli peppers samples (0.85 g) were ground and homogenized with an adsorbent powder (0.5 g) "Miyazaki Hydro-Protect", 100 mg of the mixture were placed in the extraction vessel (SFE unit). The purpose of the absorbent powder ("Miyazaki Hydro-Protect" Patented in Japan no. 3645552) was to remove the sample water content thus generating granules having dimension below 1 mm of diameter.

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2-3. Instrumentation

The SFC-MS analyses were performed on a Shimadzu Nexera UC system (Shimadzu, Japan), consisting of a CBM-20A controller, two LC-20ADXR dual-plunger parallel-flow pumps, an LC-30ADSF CO_2 pump, two SFC-30A back pressure regulator, a DGU degasser, a CTO-20AC column oven, a SIL-30AC autosampler, an LCMS-8050 triple quad mass spectrometer equipped with an APCI source, reported in Fig. 1. The entire system was controlled by the LabSolutions™ ver. 5.80.

2-4. Supercritical fluid extraction conditions

Solvent	: A) CO_2
	B) Methanol
Flow rate	: 2 mL/min
Extraction	: 0-3 min. Static mode (B. Conc. 10 %)
	3-4 min. Dynamic mode (B. Conc. 0 %)
Extraction vessel temperature	: 80 ℃
BPR	: 15 MPa

2-5. Supercritical fluid chromatography conditions

Column	: Fused Core C30,
	150 mm L.× 4.6 mm I.D., 2.7 µm
Mobile phase	: A) CO_2
	B) Methanol
Gradient program	: 4-6 min 0 % B, 6-21 min, 0-80 % B
Flow rate	: 2 mL/min
Make-up	: Methanol (1 mL/min)
BPR	: 15 MPa
MS Acquisition mode (APCI)	: SCAN (+)/(-); SIM (+)/(-); MRM (+)/(-)

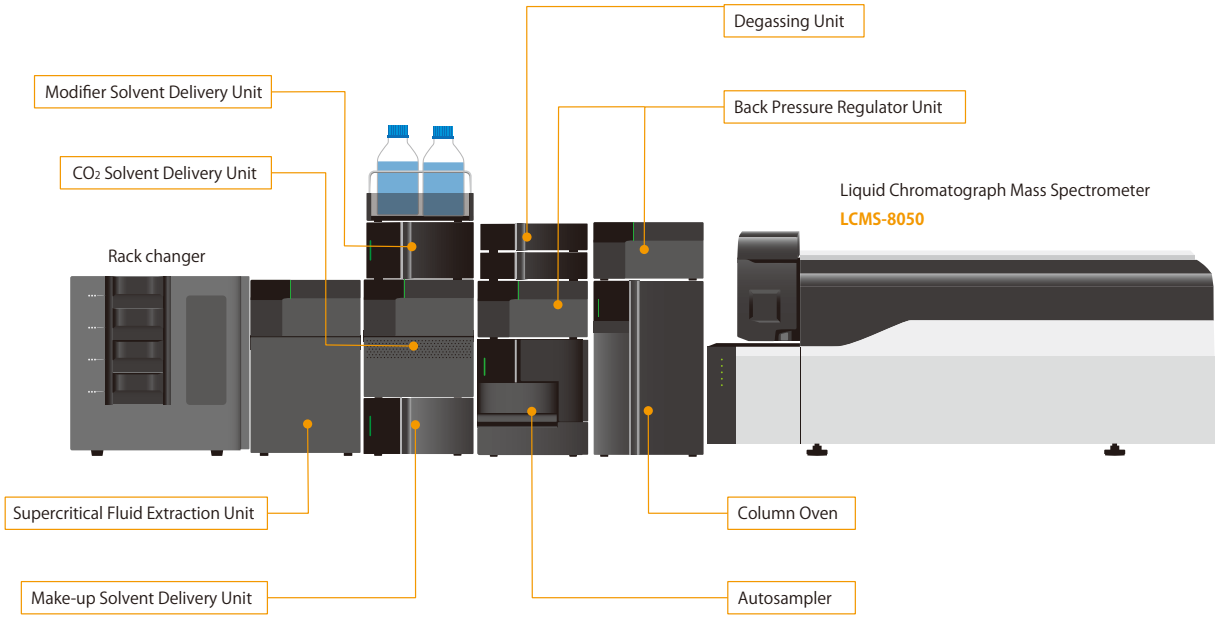

Fig. 1 Scheme of the Nexera UC instrument

3. Results and discussion

A limited number of reports on apocarotenoids in food are available in the literature. Considering the worldwide use of chilli peppers and their economic value, it has been here considered important to investigate the native apocarotenoids profile of different chilli peppers cultivars providing in this study, qualitative and also selected quantitative, not previously reported data.

In this study a further step on apocarotenoids investigation in chilli peppers cultivars was achieved; the Nexera UC instrument was employed to investigate the qualitatively native apocarotenoids profile in seventeen different chilli peppers cultivars. Totally 19 free apocarotenoids and 8 apocarotenoids fatty acid esters were identified, by the application of an online SFE-SFC-MS/MS methodology, as shown in Table 1. Interestingly, different ε apoluteinals and 4-oxo-apo-β-carotenals were detected in Capsicum species also for the first time and, to the best of authors knowledge, in any food matrix.

β-citraurin was not detected in any of the C. baccatum cultivars and was mainly present in the C. chinense cultivars. β-Apo-8′-carotenal was detected in all the C. baccatum cultivars and was also the most represented apocarotenoid among the investigated cultivars, being present in twelve out of seventeen cultivars; on the contrary Apo-14′- and Apo-15′-capsorubinal were detected only in the Jalapeno cultivar. The Habanero Red Savina cultivar showed the highest presence of different apocarotenoids.

Table 1 Qualitative profile of the investigated samples

	Baccatum			Annum					Chinese								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
β-Citraurin						✓			✓	✓			✓		✓		✓
Apo-10′ -Zeaxanthinal		✓								✓						✓	
Apo-14′ -Zeaxanthinal		✓							✓	✓							
Apo-15′ -Zeaxanthinal		✓															
Apo-8′ -Carotenal	✓	✓	✓		✓		✓		✓	✓	✓	✓	✓		✓		✓
Apo-10′ -Carotenal		✓							✓	✓			✓	✓	✓	✓	✓
Apo-12′ -Carotenal	✓	✓								✓	✓		✓		✓	✓	✓
Apo-14′ -Carotenal	✓	✓				✓		✓							✓		✓
Apo-8′ -Luteinal										✓							
Apo-10′ -Luteinal			✓		✓	✓		✓									
Apo-12′ -Luteinal			✓									✓					
Apo-14′ -Luteinal	✓		✓			✓				✓							
Apo-15′ -Luteinal			✓		✓												
Apo-10′ -Capsorubinal					✓					✓				✓			
Apo-12′ -Capsorubinal		✓		✓	✓			✓	✓	✓				✓			
Apo-14′ -Capsorubinal						✓											
Apo-15′ -Capsorubinal						✓											
Apo-12′ -Canthaxanthinal																	✓
Apo-14′ -Canthaxanthinal							✓										✓
Apo-10′ -Zeaxanthinal-C4:0	✓		✓	✓	✓							✓					
Apo-10′ -Zeaxanthinal-C10:0				✓													
Apo-10′ -Zeaxanthinal-C12:0	✓	✓	✓	✓	✓	✓			✓			✓	✓	✓	✓	✓	✓
Apo-10′ -Zeaxanthinal-C14:0									✓			✓	✓		✓		✓
Apo-8′ -Zeaxanthinal-C6:0							✓		✓	✓		✓					
Apo-8′ -Zeaxanthinal-C10:0													✓				
Apo-8′ -Zeaxanthinal-C12:0	✓	✓		✓	✓	✓						✓	✓	✓	✓	✓	✓
Apo-8′ -Capsorubinal-C12:0																	✓

The apocarotenoid fatty acid esters were mainly esters of Apo-10′- and Apo-8′-zeaxanthinal; lauric acid esters with both Apo-10′- and Apo-8′-zeaxanthinal were the most represented apocarotenoid esters among all the investigated cultivars, whereas caproic fatty acid esters of Apo-10′- and Apo-8′-zeaxanthinal were only detected, respectively in Banana peppers and Naga Morich cultivars; Apo-8′-capsorubinal lauric acid ester was only detected in Trinidad Scorpion Moruga. β-Apo-8′-carotenal and Apo-8′- zeaxanthinal (β-citraurin) were quantified and the obtained values were reported in Table 2.

Conclusions

The conducted research demonstrated the occurrence of different apocarotenoids and apocarotenoid fatty acid esters in 17 Capsicum varieties. The possible occurrence of different prevailing carotenoids oxidative cleavages pathways taking place in the different cultivars has been observed. Different ε-apoluteinals and 4-oxo-apo-β-carotenals were detected in Capsicum species for the first time using the Nexera UC instrument.

Table 2 Quantitative profile of the investigated samples expressed as mg in 100 g

Sample No.	β-Citaraurin	apo-8′ -Carotenal
1	9.2	5.2
2	≦LOD	6.8
3	≦LOD	4.9
4	≦LOD	≦LOD
5	≦LOD	14.3
6	9.0	12.3
7	≦LOD	6.3
8	≦LOD	≦LOD
9	12.6	6.1
10	61.0	33.6
11	≦LOD	5.1
12	≦LOD	5.4
13	8.2	5.0
14	≦LOD	≦LOD
15	14.2	5.0
16	≦LOD	≦LOD
17	8.7	5.7

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