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Technical Report

Characterization of 16 *Capsicum* Varieties by Evaluation of Their Carotenoid Profile by SFE-SFC-MS/MS

SFE-SFC-MS/MS analysis of carotenoids in *Capsicum* samples

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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 carotenoids characterization of 16 *Capsicum* varieties was here reported. 41 compounds were extracted and identified by the developed SFE-SFC-MS/MS methodology in less than 20 min, including free carotenoids, carotenoids monoesters, carotenoids diesters and chlorophylls in a very fast, and efficient way.

Keywords: SFE-SFC-MS/MS, *Capsicum*, Carotenoids

1. Introduction

The genus *Capsicum*, which originates from tropical and humid zones of Central and Southern America, belongs to the *Solanaceae* family and includes peppers of important economic value. *Capsicum* are one of the oldest and most popular vegetables and spices in the world. Several *Capsicum* species exist, three of which are widely spread and have a hot and pungent berry: *Capsicum annuum*, *Capsicum frutescens* and *Capsicum chinense*. The ripe fruits of the different varieties of peppers have been traditionally widely used as natural food colourants. Although generally, the colour of each *Capsicum* variety is variable, starting from green, yellow or white for the unripe fruit, and turning to red, dark red, brown and sometimes almost black in the ripe stage, ripe pepper (*Capsicum* sp.) fruits can display a range of colours from white to deep red.

Analytical methods for detecting carotenoids in food samples are mainly based on liquid chromatography approaches with relatively long analytical times and considerable organic solvent consumption. The C30 stationary phase is currently the most used phase for HPLC carotenoids separations and although UHPLC technology is available, only one report is available in the literature on the UHPLC technology applied to the monodimensional carotenoid esters analysis using a sub 2 µm particles of C18 stationary phase and only one report is available in the literature on the carotenoids separation using specific C30 stationary phase with sub-2 micron particles. Although recently, supercritical fluid chromatography (SFC) coupled to mass spectrometry has gained attention as a green, fast and useful technology applied to the carotenoids analysis, and few recent reports are available on the supercritical fluid extraction of carotenoids, only one report is available in the literature on the direct online extraction and determination of carotenoids, by a supercritical fluid extraction-supercritical fluid chromatography-mass spectrometry (SFE-SFC-MS) methodology. The aim of this research was focused on the development of an online 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 carotenoids characterization of 16 *Capsicum* varieties, in order to discriminate the different varieties.

2. Experimental

2-1. Samples

16 Fresh samples belonging to the genus *Capsicum* were kindly provided by Peperita «Azienda agricola» (Livorno, Italy) namely: Aji *Capsicum Baccatum* (S1), Banana Pepper *Capsicum Annum* (S2), Erotico *Capsicum Baccatum* (S3), Caienna Impala *Capsicum Annum* (S4), Jalapeño *Capsicum Annum* (S5), Scotch Bonnet *Capsicum Chinense* (S6), Habanero Red Savina *Capsicum Chinense* (S7), Habanero Fatal *Capsicum Chinense* (S8), Habanero Chocolate *Capsicum Chinense* (S9), Jimmy *Capsicum Baccatum* (S10), Naga Morich *Capsicum Chinense* (S11), Naga Yellow *Capsicum Chinense* (S12), Naga Chocolate *Capsicum Chinense* (S13), Terenzio *Capsicum Annum* (S14), Trinidad Scorpion *Capsicum Chinense* (S15), Trinidad Scorpion Moruga Yellow *Capsicum Chinense* (S16).

2-2. Sample preparation

Samples (1 g) were ground by using the ultra-turrax T 25 and homogenized with an adsorbent powder (1 g) "Miyazaki Hydro-Protect," Patented in Japan no. 3645552, and placed in the extraction vessel in the SFE unit.

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A 0.2 mL extraction vessel was used (the ID of the extraction chamber was 6 mm and the lenght was 12 mm), loaded with 100 mg of sample/adsorbent (considering the dilution factor a final amount of 50 mg of sample was used).

2-3. Instrumentation (Shimadzu)

The SFE-SFC-MS analyses were performed on a Shimadzu Nexera-UC system consisting of a CBM-20A controller, an SFE-30A module for supercritical fluid extraction, two LC-20ADXR dual plunger parallel-flow pumps, an LC-30ADSF CO2 pump, two SFC-30A back pressure regulator, a DGU degasser, a CTO-20AC column oven, a SIL-30AC autosampler, an LCMS-8050 mass spectrometer equipped with an APCI source. The entire system was controlled by the LabSolutions ver. 5.80.

2-4. Supercritical fluid extraction conditions

Solvent	: A) CO_2
	B) MeOH
Flow rate	: 2 mL min^{-1}
Extraction	: 0-3 min. Static mode (B.Conc. 10%)
	3-4 min. Dynamic mode (B.Conc. 0%)
Extraction vessel temperature	: 80°C
BPR	: 15 MPa
Make-up	: MeOH

2-5. Supercritical fluid chromatography conditions

Column	: C30, 150 mm L. × 4.6 mm I.D., 2.7 µm
Mobile phase	: A) CO_2
	B) MeOH
Gradient program	: 0-2 min, 0% B; 2-17 min 80% B
Flow rate	: 2 mL min^{-1}
Make-up	: MeOH
Column temperature	: 35°C
BPR	: 15 MPa
MS Acquisition mode (APCI)	: SCAN (+)/(-); SIM (+)/(-); MRM

2-6. Software

LabSolutions software ver. 5.80

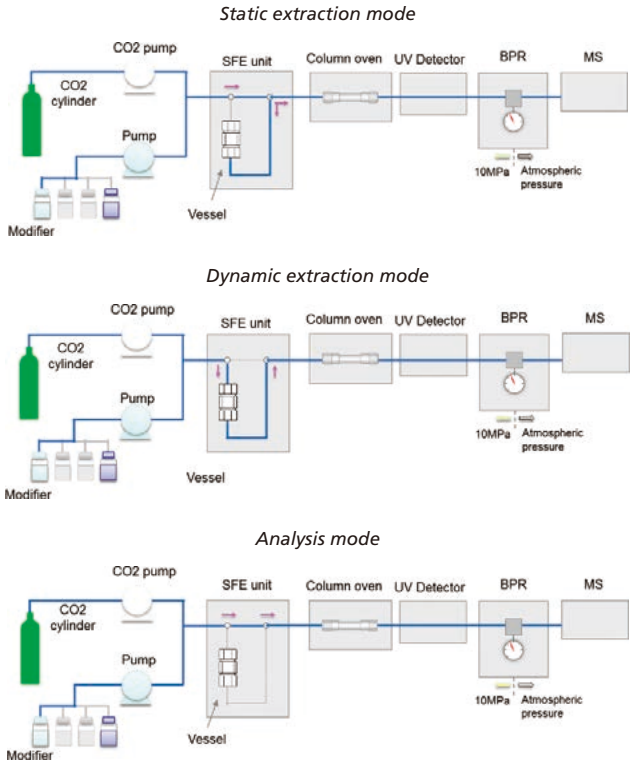

Fig. 1 Scheme of the SFE-SFC instrument.

3. Results and discussion

The study of intact carotenoids (samples without saponification) composition could be a useful tool to increase the certainty about the carotenoids naturally found in the various matrices and the relationships between them, and could be also used to establish authenticity markers. In this contribution an SFE-SFC-MS/MS platform for the native carotenoids characterization of 16 *Capsicum* varieties was developed. In Table 1 are shown the carotenoids and chlorophylls composition of the cultivars of chilli peppers investigated. The correct characterization of these compounds is necessary to obtain reliable compositional data for realistic and valuable conclusions in nutritional studies.

Here we report a developed (SFE-SFC-MS/MS) method for the direct identification of the native carotenoid composition in various Capsicum cultivars, belonging to three species (*C. chinense, C. annuum, C. frutescens*).

Table 1 Carotenoids and chlorophylls composition of the 16 cultivars [a] of chilli peppers investigated.

N°	Compound	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16
1	(13Z)-cis-β-Cryptocapsin			×	×	×	×		×	×	×			×	×		×
2	β-Carotene-5,6-epoxide			×	×			×	×	×	×	×		×	×	×	×
3	β-Carotene-5,8-epoxide					×				×	×						
4	α-Cryptoxanthin				×	×	×	×	×	×		×		×			
5	(13Z)-cis-Cryptocapsin				×	×	×			×		×	×				
6	Cryptocapsin			×	×		×				×			×		×	
7	Cryptoxantin-5,6-epoxide			×													
8	(13Z)-cis-β-Carotene				×			×	×		×	×		×	×		
9	(9Z)-cis-α-Carotene			×							×						×
10	β-Carotene			×	×			×	×		×	×		×	×		×
11	α-Carotene			×	×			×	×		×	×		×	×		×
12	Antheraxanthin								×	×	×	×	×	×	×	×	
13	cis-Capsanthin			×													
14	Capsanthin			×	×	×	×	×		×	×	×		×	×	×	×
15	Chlorophyll a		×	×		×	×										
16	Chlorophyll b		×	×		×	×										
17	Lutein		×	×	×	×	×	×		×			×	×	×	×	×
18	Zeaxanthin		×	×	×	×	×	×	×	×			×	×	×	×	×
19	Capsanthin-5,6-epoxy-C14:0			×	×									×	×	×	×
20	Capsorubin			×					×		×						×
21	Luteoxanthin	×			×			×	×								×
22	Pheophytin a			×								×		×			
23	Phytoene	×			×		×	×				×		×			×
24	β-Cryptoxanthin		×	×	×		×	×		×		×			×		×
25	Phytofluene	×		×	×	×	×	×		×		×		×	×		×
26	Capsanthin-C12:0				×						×			×		×	
27	Capsanthin-C16:0			×											×		
28	Zeaxanthin-C12:0			×								×			×		
29	Antheraxanthin-C14:0				×							×				×	
30	Capsanthin-C14:0				×											×	
31	cis-Capsanthin-C14:0				×							×					
32	Lutein-C14:0			×						×			×		×	×	
33	Zeaxanthin-C14:0			×						×			×		×	×	
34	Cryptocapsin-C14:0			×												×	
35	cis-Capsanthin-C12:0				×												
36	Antheraxanthin-C12:0										×		×			×	
37	Capsanthin-C14:0, C14:0								×					×			
38	Capsanthin-C14:0, C16:0													×			
39	Zeaxanthin-C14:0, C16:0													×			
40	Capsanthin-C12:0, C16:0											×					
41	Capsanthin-C12:0, C14:0															×	

[a] Aji *Capsicum Baccatum* (S1), Banana Pepper *Capsicum Annum* (S2), Erotico *Capsicum Baccatum* (S3), Caienna Impala *Capsicum Annum* (S4), Jalapeño *Capsicum Annum* (S5), Scotch Bonnet *Capsicum Chinense* (S6), Habanero Red Savina *Capsicum Chinense* (S7), Habanero Fatal *Capsicum Chinense* (S8), Habanero Chocolate *Capsicum Chinense* (S9), Jimmy *Capsicum Baccatum* (S10), Naga Morich *Capsicum Chinense* (S11), Naga Yellow *Capsicum Chinense* (S12), Naga Chocolate *Capsicum Chinense* (S13), Terenzio *Capsicum Annum* (S14), Trinidad Scorpion *Capsicum Chinense* (S15), Trinidad Scorpion Moruga Yellow *Capsicum Chinense* (S16).

Compounds were identified by comparison with the available standards, by their MS spectra recorded in both positive and negative APCI ionisation modes and in multiple reaction monitoring. Typical carotenoids MS fragmentation pattern in APCI ionisation mode showed, in the case of the carotenoids esters, the losses of the corresponding esterified fatty acids from the pseudomolecular ion, and in the case of free carotenoids, the losses of water molecule or toluene moiety. In total, 41 carotenoids have been identified and considerable variation in carotenoid composition was observed among the various cultivars investigated, in terms of presence or absence of certain carotenoids.

As it can be observed from Table 1 the various carotenoids were present in the various cultivars with noticeable difference.

In fact the number of identified carotenoids ranged from 4 for the Aji *Capsicum Baccatum* species to 23 for the Erotico *Capsicum Baccatum* species, with an average of 13 compounds.

Interestingly, as previously reported the absence of β-carotene in some pepper cultivars. As expected, capsanthin was present in all the cultivars investigated except for the yellow coloured Aji *Capsicum Baccatum*, Banana Pepper *Capsicum Annum*, Habanero Fatal *Capsicum Chinense*, Naga Yellow *Capsicum Chinense* Cryptoxantin-5,6-epoxide and cis-Capsanthin were found only in the Erotico *Capsicum Baccatum* sample.

Only in the sample Caienna Impala *Capsicum Annum* was detected the cis-Capsanthin-C12:0, while the Capsanthin-C12:0, C16:0 was identified in the sample Naga Morich *Capsicum Chinense*, and Capsanthin-C12:0, C14:0 was detected in the Trinidad Scorpion *Capsicum Chinense* sample.

The Naga Chocolate *Capsicum Chinense* sample shown the greatest variety of di-esters compounds (Capsanthin-C14:0, C14:0, Capsanthin-C14:0, C16:0 and Zeaxanthin-C14:0, C16:0).

4. Conclusions

The carotenoids composition of 16 different varieties of Capsicum species, not previously fully investigated for their native carotenoids profile, was here directly investigated by an SFE-SFC-MS methodology. In total, 41 carotenoids have been identified and considerable variation in carotenoid composition was observed among the various cultivars investigated.

The developed SFE-SFC-MS method provides a platform for the online extraction and separation of relatively non polar compounds that was here applied to the carotenoids analysis. The on-line nature of the system, greatly reduce the analytical times in a very fast, efficient and green way and that could be also applied to the separations of other phytochemicals in differents complex matrices. In fact, considering both extraction process (compared to the traditional solid/liquid extraction, which may required a few hours) and chromatographic run, the developed method run-time was just less than 20 min.

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