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Technical Report

On-line extraction and determination of targeted carotenoids from habanero red (Capsicum Chinese)

SFE-SFC: sample preparation and measurement

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Abstract:

The on-line coupling between supercritical fluid extraction (SFE) and supercritical fluid chromatography (SFC), generate a powerful tool for the automatic extraction and detection of target compounds from food matrices. The on-line nature of the system, compared to off-line approaches, improves run-to-run precision, enables the setting of batch-type applications, and reduces the risks of sample contamination. Supercritical carbon dioxide (CO_2) presents unique characteristics, which make it an excellent solvent, it shows a relatively high density and consequently, a high solvation power. Finally, the QqQ MS detector due to its high sensitivity and selectivity allows the identification and structural analysis of targeted and untargeted compounds. The analytical potential of the system described are shown in a proof of principle study involving the carotenoids study from food samples.

Keywords: SFE; SFC; Carotenoids; QqQ MS.

Introduction

Carotenoids are natural pigments synthesized by plants and some microorganisms. Humans and animals are not able to synthesize carotenoids de novo and they need to acquire them through their diet. Carotenoids and their derivatives are versatile isoprenoids based on a C40 tetraterpenoid skeleton and play a vital role in plants and animals. The most significant aspect of carotenoids in our diet is the antioxidant and provitamin A activity, and also the color that they impart to our food [1]. Carotenoids are usually divided into two groups: hydrocarbons, composed of only carbon and hydrogen, e.g., lycopene and β-carotene (carotenes) and oxygenated compounds (xanthophylls), which are oxygenated and may contain epoxy, carbonyl, hydroxy, methoxy or carboxylic acid functional groups.

Fig. 1 SFE-SFC-QqQ MS instrumentation

Examples of xanthophylls are violaxanthin (epoxy), canthaxanthin (oxo), zeaxanthin (hydroxy), spirilloxanthin (methoxy) and torularhodin (carboxylic acid). Moreover, must be considered that in nature two different forms of carotenoids can be found, respectively as free carotenoids, and in the case of oxygenated components in a more stable form esterified with fatty acids. The main feature of the carotenoid structure is the long system of alternating single and double bonds forming a conjugated system in which the π-electrons are delocalized along the entire polyene chain. In the present work the carotenoids composition of a sample belongs to the Solanaceae family, specifically Capsicum was analyzed [2].

Capsicum, originates from tropical and humid zones of Central and Southern America, is one of the oldest and most popular vegetable and spice in the world, and includes peppers. The research is focused on the development of an on-line method coupling supercritical fluid extraction (SFE) and supercritical fluid chromatography (SFC) for the detailed characterization of the carotenoid composition of a habanero red sample. The on-line nature of the system, compared to off-line approaches, improves run-to-run precision, enables the setting of batch-type applications, and reduces the risks of sample contamination. Supercritical carbon dioxide (CO_2) presents unique characteristics, which make it an excellent solvent. CO_2 shows a relatively high density and consequently, a high solvation power. It presents low viscosity and high diffusion coefficient, which allow fast extraction. From the environmental point of view, the use of supercritical CO_2 for both purposes, SFE and SFC, greatly reduces the use of organic solvents. The density of the supercritical fluid can be easily handled by changing its pressure and/or temperature, changing the solvent strength. The same considerations can be made regarding the SFC, thus obtaining rapid analysis characterized by high resolution. Finally, the MS detector due to its high sensitivity and selectivity allows the identification and structural analysis of targeted and untargeted compounds.

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Experimental

Samples and sample preparation

In the on-line supercritical fluid extraction supercritical fluid chromatography (SFE-SFC) approach, the habanero sample (1 g) was grinded by using the ultra-turrax and homogenized with an absorbent powder (1 g) and placed in the extraction vessel in the SFE unit. A 0.2 mL extraction vessel was used, loaded with 100 mg of sample/adsorbent. Supercritical CO_2 was then introduced into the vessel, where extraction conditions were optimized with respect to pressure and temperature. After extraction in the SFE unit, the sample-containing CO_2 was directed in the SFC flow line for performing the following chromatographic analysis.

Instrumentation (Shimadzu)

SFE-SFC-MS analyses were performed on a Shimadzu Nexera-UC system (Fig. 1), 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 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. The entire system was controlled by the LabSolutions ver. 5.8.

Chromatographic method

SFE: Solvent: (A) CO_2; (B) CH_3OH; Gradient: 50 % B; 1.1 min 0 % B; Flow rate: 0-1 min 2 mL/min; 1-4 min 3 mL/min; 4-20 min 2 mL/min; Extraction mode: 0-3 min static mode, 3-4 dynamic mode; Extraction vessel temperature: 40, 50, 50, 60, 70 and 80 °C;
SFC: Analytical column: Fused Core C30, 150 mm × 4.6 mm, 2.7 µm d.p.
Mobile phase: (A) CO_2; (B) CH_3OH; (make-up) CH_3OH; Gradient: 0-2 min, 0% B; 2-10 min 40 % B; 10-13 min 40% B; Flow rate: 2 mL/min; Column oven: 35 °C; Back Pressure regulator A: 150 bar; MS Acquisition mode: SCAN (+)/(-); SIM (+)/(-); MRM (+);

Table 1 MS APCI (+) and APCI (-) information for the identification of the carotenoids and chlorophylls analysed in this work.

ID	Identification	MS data APCI (+) e (-)
1	Luteoxanthin	601, 583 (+); 600 (-)
2	Antheraxanthin	585, 567 (+); 584 (-)
3	cis-Capsanthin	585, 567,(+); 584 (-)
4	(13Z)-cis-Cryptocapsin	569 (+); 568 (-)
5	Chlorophyll b	907 (+); 906 (-)
6	Lutein	551 (+); 568 (-)
7	Capsanthin	585, 567, 479 (+); 584 (-); 478 (-)
8	Zeaxanthin	569 (+); 568 (-)
9	Chlorophyll a	893 (+); 892 (-)
10	(13Z)-cis-β-Cryptoxanthin	553 (+); 552 (-)
11	Cryptocapsin	569 (+); 568 (-)
12	Phytoene	545 (+); 544 (-)
13	Cryptoxantin-5,6-epoxide	569 (+); 568 (-)
14	α-Cryptoxanthin	553 (+); 552 (-)
15	(9Z)-cis-α-Carotene	537 (+); 536 (-)
16	β-Cryptoxanthin	553 (+); 552 (-)
17	Phytofluene	543 (+); 542 (-)
18	β-Carotene-5,6-epoxide	553 (+); 552 (-)
19	β-Carotene-5,8-epoxide	553 (+); 552 (-)
20	(13Z)-cis-β-Carotene	537 (+); 536 (-)
21	α-Carotene	537 (+); 536 (-)
22	cis-Capsanthin-C12:0	767, 567 (+); 766 (-)
23	cis-Capsanthin-C12:0	767, 567 (+); 766 (-)
24	Antheraxanthin-C14:0	795, 567 (+); 794 (-)
25	β-Carotene	537 (+); 536 (-)
26	Capsanthin-5,6-epoxy-C14:0	811, 583 (+); 810 (-)
27	Pheophytin a	872 (+); 871 (-)
28	cis-Capsanthin-C14:0	795, 567 (+); 794 (-)
29	Lutein-C14:0	779, 551 (+); 778 (-)
30	Capsanthin-C12:0	767, 567 (+); 766 (-)
31	Zeaxanthin-C12:0	751, 551 (+); 750 (-)
32	Capsanthin-C14:0	795, 567 (+); 794 (-)
33	Zeaxanthin-C14:0	779, 551 (+); 778 (-)
34	Capsanthin-C16:0	823, 567 (+); 822 (-)
35	Zeaxanthin-C16:0	807, 551 (+); 806 (-)
36	β-Cryptoxanthin-C12:0	735, 535 (+); 734 (-)
37	Cryptocapsin-C14:0	779, 551 (+); 778 (-)
38	β-Cryptoxanthin-C14:0	763, 535 (+); 762 (-)
39	Cryptocapsin-C16:0	807, 551 (+); 806 (-)
40	β-Cryptoxanthin-C16:0	791, 535 (+); 790 (-)
41	Capsanthin-C12:0, C14:0	977, 777, 749, 549; 976 (-)
42	Zeaxanthin-C12:0, C12:0	933, 733, 533; 932 (-)
43	Capsorubin-C14:0,C14:0	1021, 793, 565; 1020 (-)
44	Zeaxanthin-C12:0, C14:0	961, 733, 533 (+); 960, 760 (-)
45	Capsanthin-C14:0, C14:0	1005, 777, 549 (+); 1004 (-)
46	Capsorubin-C14:0,C16:0	1049, 821, 793, 565 (+); 1048 (-)
47	Capsanthin-C12:0, C16:0	1005, 805, 749, 549 (+); 1004 (-)
48	Zeaxanthin-C14:0, C14:0	989, 761, 533 (+); 988, 760 (-)
49	Capsanthin-C14:0, C16:0	1033, 805, 777, 549 (+); 1032 (-)
50	Zeaxanthin-C14:0, C16:0	1017, 789, 761, 533 (+); 1016 (-)
51	Capsanthin-C16:0, C16:0	1061, 805, 549 (+); 1060 (-)
52	Zeaxanthin-C16:0, C16:0	1045, 789, 533 (+); 1044, 788 (-)

Results and discussion

An SFE-SFC-APCI-QqQ MS method for the direct identification of the native carotenoid composition in habanero red (Capsicum chinense) sample was developed. Compounds were identified by comparison with the available standards, by using the elution order, and by their MS spectra recorded in both positive and negative APCI ionization modes, the possibility of rapid switchover between negative and positive ionisation mode in the APCI probe allowed us to collect more qualitative information about a sample in a single run, with quasi-molecular ion species dominating the MS spectrum in one case (negative mode), or abundant fragmentation in the other (positive mode).

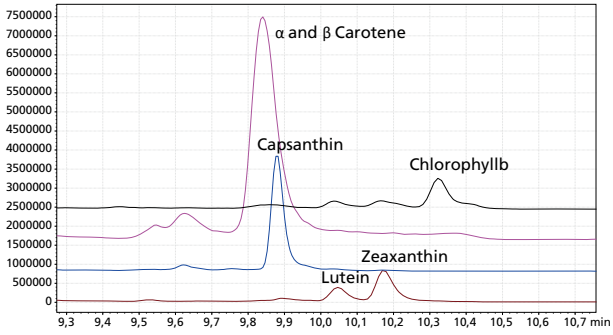

Fig. 2 Expansion of the free carotenoids zone acquired in SIM mode of a red habanero sample.

Moreover three components were pinpointed by using the multiple reaction monitoring (MRM), namely Capsanthin, Lutein, and Zeaxanthin. The SFE has been optimized using different temperatures, starting from 40 °C up to 80 °C using an increase of 10 degrees. Multiple extractions, until depletion, were performed on the same sample, in order to evaluate the extraction yield. For all the compounds considered, in the fourth extraction only some traces were eventually present. Totally 21 analytes were extracted and identified, considering the average of the first extraction among all the compounds, it can be noted an increasing trend in the extraction yield, by increasing temperature: 40 °C average 41.8 % (min. value 35.9 %, max value 51.2 %), 50 °C average 46.4 % (min. value 38.0 %, max value 55.0 %), 60 °C average 46.7 % (min. value 39.5 %, max value 53.4 %), 70 °C average 47.1 % (min. value 38.3 %, max value 54.8 %), and 80 °C 48.6 °C (min. value 37.4 %, max value 65.4 %).
In Table 1 are reported the MS [APCI (+) and APCI (-)] ions used for the identification of the carotenoids and chlorophylls. In Fig. 2 is reported an expansion of the chromatogram (SIM) with peak identifications of the free carotenoids.

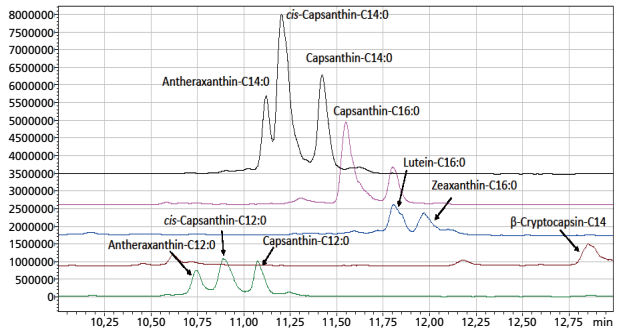

Fig. 3 Expansion of the carotenoids mono-ester zone acquired in SIM mode of a red habanero sample.

As can be observe six different components were identified, respectively: α Carotene, β Carotene, Capsanthin, Lutein, Zeaxanthin, and Chlorophyll b. α and β Carotene appear to be co-eluted, due to their high amount. In Fig. 3 is reported the expansion (SIM) of mono-ester carotenoids area of the chromatogram. Ten different components were identified: Antheraxanthin-C12:0 and C14:0, Capsanthin-C12:0, C14:0, and C16:0, cis-Capsanthin-C12:0 and C14:0, Lutein-C16, Zeaxanthin-C16:0, and β-Cryptocapsin.

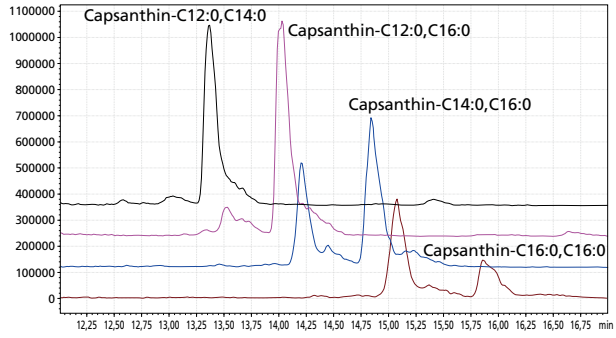

Fig. 4 Expansion of the carotenoids di-ester zone acquired in SIM mode of a red habanero sample.

In Fig. 4 is reported the expansion (SIM) of di-ester carotenoids area of the chromatogram. Four components were identified belonging to the Capsanthin family: Capsanthin-C12:0, C14:0; C12:0, C16:0; C14:0, C16:0; and C16:0, C16:0.

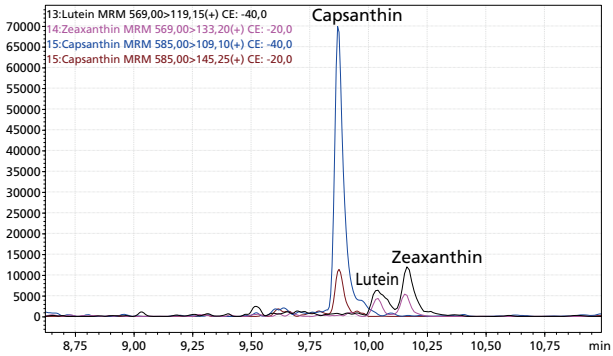

Fig. 5 Expansion of the same chromatogram zone reported in Fig. 1 but acquired by MRM.

Finally, to evaluate the performance of the MS system, the MRM acquisition mode was used simultaneously. In Fig. 5 is reported the expansion of the same area reported in Fig. 2, showing the MRM acquisition for Capsanthin (585<109 CE 40 eV and 585<145 CE 20 eV), Lutein (569<119 CE 40 eV and 569<133 CE 20 eV), and Zeaxanthin (569<119 CE 40 eV and 569<133 CE 20eV).

Conclusions

The on-line nature of the system, compared to off-line approaches, drastically reduces the extraction time (compared to the traditional solid/liquid extraction, which required about a couple of hours), reduces the analysis run time, reduces the risks of sample contamination, improves run-to-run precision, and enables the setting of batch-type applications. In fact considering both extraction process and chromatographic run, the developed method run-time is 18 min.

References

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[2] S.M. Rivera, R. Canela-Garayoa. Journal of Chromatography A 1224 (2012) 1–10.

First Edition: January, 2024

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3655-12310-PDFIK