Elucidation of carotenoids in microalgae formulations by APCI-DI-MRMS

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Introduction

Among the natural matrices rich in bioactive compounds, microalgae represent one of the most promising matrices [1]. These microorganisms are a source of various biologically active molecules, including amino acids, polyunsaturated fatty acids, minerals, proteins and pigments. Spirulina-based products are used by athletes as anti-fatigue and amino acid supply, and for their anti-aging detoxifying and antioxidant properties in cosmetics. The antioxidant potential of Spirulina is partially attributed to the high content of natural pigments, especially carotenoids, which are also recognized for having numerous healthy benefits. Profiling of pigment in this species has been only partially described yet. Given that the Spirulina pigment fraction is highly complex, conventional LC-MS based methods suffer from low separation efficiency of very complex mixtures as well as long analysis time and limited mass accuracy, which can result in inaccurate and incorrect compound identification. In this regard, the objective of this study was the development of a combined platform for qualitative and quantitative characterization of Spirulina pigments in different dietary supplements. In this study ultra-high mass resolution of Magnetic Resonance Mass Spectrometry (MRMS) for qualitative profiling of the extract was applied.

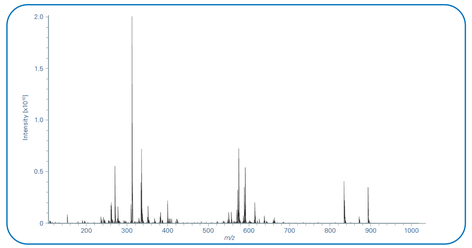

Fig. 1: Broad band APCI-MRMS spectrum of a Spirulina powder extract using positive ion mode

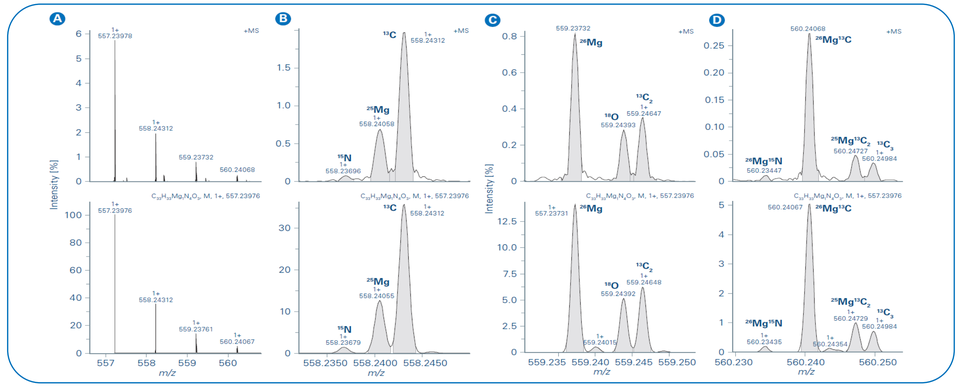

Figure 2: Zoom-in of broad band spectrum at m/z 557 of compound Pyro chlorophyllide a ($C_{33}H_{32}MgN_4O_3$) and IFS pattern. Measured spectrum in top and simulated spectrum in bottom, A) zoom-in at m/z 557 of isotopic pattern, B) A+1 pattern, C) A+2 pattern, D) A+3 pattern.

Methods

350 mg of Spirulina powder (tablets were prior pulverized in a mortar) were treated with 50 mL of ethanol fortified with 20 µg/mL. Sample was subjected for 15 min in an ultrasonic bath, then the suspension was stirred for 30 min at room temperature. Supernatant was removed, and the pellet was retreated following the same protocol another four times. Supernatants were pooled and lyophilized.

Data were acquired on a solariX XR 7T (Bruker Daltonik GmbH, Bremen, Germany) equipped with an Apollo II APCI ion source. Samples (10 µg/mL in methanol) were infused into the ion source at 50 µL/min. 200 single scans were added. Spectra were acquired with 8 million data points (8M) resulting in a mass resolution of 700.000 @ m/z 400. Five measurement replicates were carried out of each sample.

Peak alignment and tentative identification of compounds was based on accurate mass measurement. Spectra were loaded in MetaboScape 4.0 (Bruker Daltonik GmbH, Bremen, Germany) for feature extraction and database search.

Results

APCI ionization was employed, which outperformed electrospray for almost all analytes classes (data not shown). Table 1 shows the detection and tentative identification of compound in three formulations. A high number of tentatively identified compounds could be detected with respect to previous investigations of Spirulina [2]. Figure 1 reports the full APCI spectrum of a Spriulina extract. Different carotenoid classes could be identified: hydroxyl, epoxy, ketocarotenoids, carotenes and well as chlorophylls and pyro chlorophylls. Several compounds are reported here for the first time present in Spirulina (Table 1). Ultra high mass accuracy lead to highly confident identification, such as for the Pyro Chlorophyllide-a, a chlorophyll derivative (Figure 2). The molecular formula could be confirmed by isotopic fine structure (IFS) of the A+1, A+2 and A+3 pattern shown in Figure 2 b-d. Based on the identified molecular formula a possible structure of this molecules can be found using CompoundCrawler (Figure 3). A further benefit of DI-MRMS is the analysis time. A mass spectrum could be acquired in only four minutes which is much faster than conventional LC-MS experiments for detection of carotenoids.

Table 1: Detection and tentative identification of compound in three formulations

Compound	Molecular Formula	$[M+H]^+$ DI-APCI-MRMs	Mass accuracy
Apo-12-Violaxanthal[1]	$C_{25}H_{34}O_3$	383.25809	-0.05
Vaucheriaxanthin[1]	$C_{40}H_{56}O_5$	617.42010	-0.08
Diadinoxanthin[1]	$C_{40}H_{56}O_3$	583.41458	-0.02
Canthaxanthin	$C_{40}H_{52}O_2$	565.40402	-0.01
Ethyl β-apo-8'-carotenoate[1]	$C_{32}H_{44}O_2$	461.34143	-0.04
Adonirubin[1]	$C_{40}H_{52}O_3$	581.39892	0.01
Diatoxanthin[1]	$C_{40}H_{54}O_2$	567.41967	-0.02
β-Apo-8'-carotenal[1]	$C_{30}H_{40}O$	417.3152	-0.01
Hexadehydro-β,β-caroten-3-ol[1]	$C_{40}H_{50}O$	547.39347	-0.06
Rhodoxanthin[a]	$C_{40}H_{50}O_2$	563.38838	-0.04
Astaxanthin	$C_{40}H_{52}O_4$	597.39382	0.02
Antheraxanthin[1]	$C_{40}H_{56}O_3$	585.43023	-0.02
Myxoxanthophyll	$C_{46}H_{66}O_7$	731.48807	0.08
Zeaxanthin	$C_{40}H_{56}O_2$	569.43529	0.02
10-Apo-β-carotenal[1]	$C_{27}H_{36}O$	377.28389	-0.06
α-tocopherol	$C_{29}H_{50}O_2$	431.38835	0.01
Echinenone	$C_{40}H_{54}O$	551.42473	0.02
Pyro Chl b	$C_{53}H_{68}MgN_4O_4$	849.51640	-0.03
Phy a derivate	$C_{55}H_{72}N_4O_6$	869.55755	0.01
Chld b	$C_{35}H_{32}MgN_4O_6$	629.24050	0.01
Chl b	$C_{55}H_{70}MgN_4O_6$	907.55824	0.01
Pyro Chl a	$C_{53}H_{70}MgN_4O_3$	835.53711	0.01
Pyro Chld a	$C_{33}H_{32}MgN_4O_3$	557.23978	-0.04
Pyro Chld b	$C_{33}H_{30}MgN_4O_4$	571.21901	0.02
OH-Chl a	$C_{55}H_{72}MgN_4O_6$	909.53746	0.05
Protochld a	$C_{35}H_{32}MgN_4O_5$	613.22959	-0.01
13-OH-Chld a	$C_{35}H_{34}MgN_4O_6$	631.24015	0.01
Divinyl Chl a	$C_{55}H_{70}MgN_4O_5$	891.52691	0.04
Chl a	$C_{55}H_{72}MgN_4O_5$	893.54262	-0.03
Cryptoxanthin*	$C_{40}H_{56}O$	553.44040	0.01
Chld a	$C_{35}H_{34}MgN_4O_5$	615.24526	-0.04
Phy b	$C_{55}H_{72}N_4O_6$	885.55233	0.14
15-OH-Lactone-Chl a	$C_{55}H_{72}MgN_4O_7$	925.53199	0.47
Pyro Pheo b	$C_{33}H_{32}N_4O_4$	549.24967	-0.08
15-OH-Lactone-Phy a	$C_{55}H_{74}N_4O_7$	903.56328	-0.28
Chlorobactene	$C_{40}H_{52}$	533.41416	0.03
Chl a derivate I	$C_{55}H_{68}MgN_4O_5$	889.51122	0.08
Phytoene	$C_{40}H_{64}$	545.50810	-0.03
13-OH-Pheo a	$C_{55}H_{74}N_4O_6$	609.27078	-0.02
OH-Phy a	$C_{55}H_{74}N_4O_6$	887.56810	0.01
β-carotene	$C_{40}H_{56}$	537.44547	0.01
Octadehydro-β,β-carotene	$C_{40}H_{48}$	529.38288	0.03
Phy a	$C_{55}H_{74}N_4O_5$	871.57318	0.02
Pheophorbide a	$C_{35}H_{36}N_4O_5$	593.27583	0.02
Pyro Pheo a	$C_{33}H_{34}N_4O_3$	535.27037	0.01
Pyro Phy a	$C_{33}H_{72}N_4O_3$	813.56769	0.04
δ-tocopherol	$C_{27}H_{46}O_2$	403.35706	0.01
γ-tocopherol	$C_{28}H_{48}O_2$	417.37270	0.02
Phytofluene	$C_{40}H_{62}$	543.49242	0.01

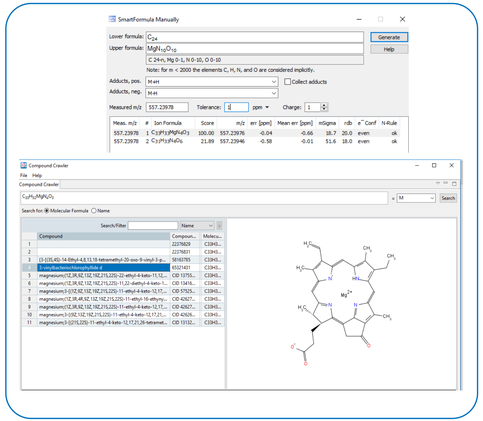

Figure 3:. Accurate mass measurement combined with isotopic fine structure resulted in Pyro chlorophyllide with a molecular formula of $C_{33}H_{33}MgN_4O_3$. Structure of could be found by CompoundCrawler.

Conclusions

- APCI combined with DI-MRMS has been proven for analysis of carotenoids in Spirulina pigment fractions.
- MRMS using ultra-high mass resolution and accurate mass as well as isotopic fine structure is a promising tool for in-depth profiling of microalgae pigments.
- DI-MRMS as tool for bio-compound is important in the nutraceutical and pharmaceutical area.

MRMS Metabolomics

References

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