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Mass Spectrometry Laboratory

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Exploring Plant Biodiversity by LC-HR-MS Chemical Fingerprint

Finger Lime fingerprint: a comparative chemical study of five different varieties

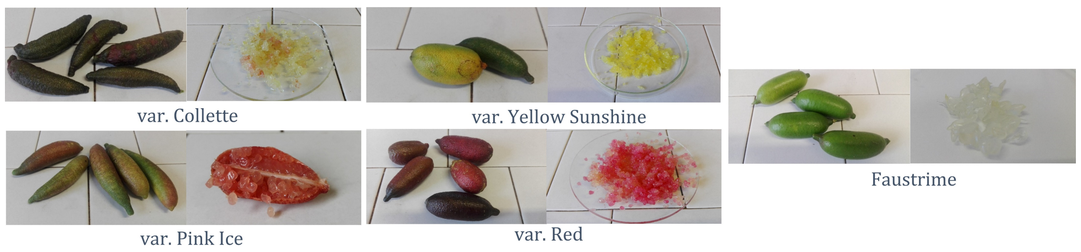


Fig.1. Finger Lime fruits.

Finger Lime is the common name of *Citrus australasica* F. Muell. (Rutaceae), a small fruit tree native to Australia. Four varieties (Collette (COL), Yellow Sunshine (YS), Pink Ice (PI), Red, and the hybrid species faustrime (FAU) (*Monocitrus australasica* x *Fortunella* sp. x *Citrus aurantifolia*) were investigated (Fig. 1). A comparative metabolomic study was carried out by ultra-high performance liquid chromatography (UHPLC) coupled to a high-resolution Orbitrap-based electrospray ionization mass spectrometer (HR-Orbitrap/ESI-MS) (Figs. 2, 3). Specialized metabolites were tentatively identified (Tab. 1), quantified (Fig. 4), and subjected to the principal component analysis (PCA) (Fig. 5).

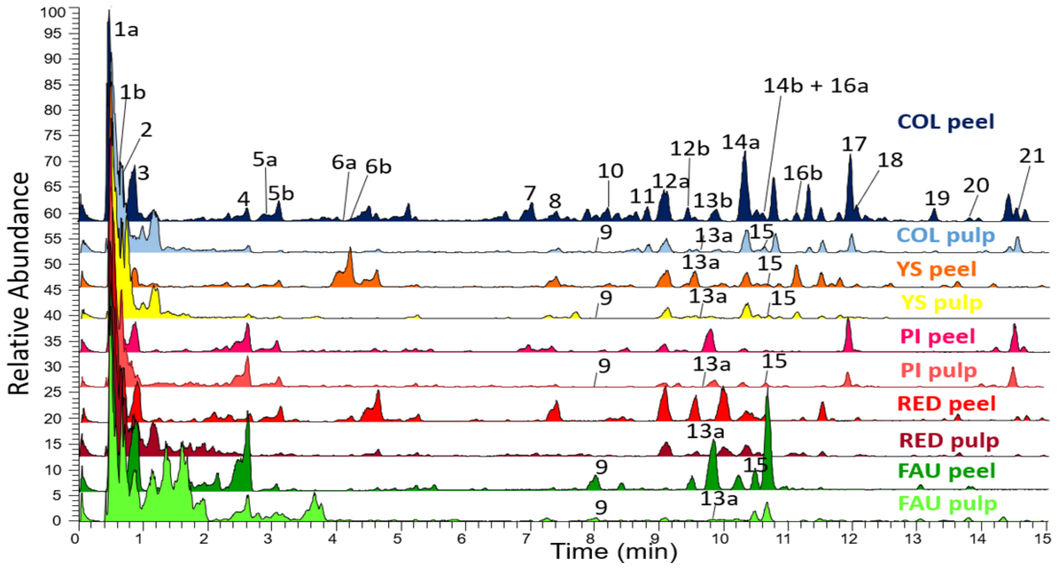


Fig.2. Peel and pulp UHPLC-HR-Orbitrap/ESI-MS profiles (negative ion mode).

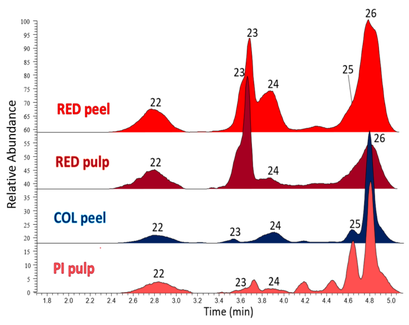


Fig.3. Anthocyanin profiles (positive ion mode).

All peels displayed a higher total phenol content than pulps. Collette peels and Pink Ice pulp showed the highest content of identified flavonoids. Collette and Red peels resulted rich in anthocyanins. The hybrid species faustrime showed the occurrence of typical *Citrus* metabolites such as (neo)eriocitrin and (neo)diosmin.

Tab.1. Identified compounds

Hydroxycinnamic acids

1a,b Caffeoylisocitric acid (isomers)
2 Caffeoylmethylisocitric acid
3 Methylisocitric acid derivatives
4 3-Hydroxy-3-methylglutaric acid derivative
5a,b *p*-Coumaroylglucoside acid (isomers)
6a,b Feruloylglucoside acid (isomers)

Flavonoids

7 Rutin
8 Quercetin glucoside
9 Neoeriocitrin/eriocitrin
10 Luteolin 7'-*O*-neohesperidoside/rutinoside
11 Kaempferol glucoside
12a,b Isorhamnetin glucoside (isomers)
13a,b Naringin/naringenin rutinoside
14a,b 3-Hydroxy-3-methylglutaryl isorhamnetin glucoside (isomers)
15 Neodiosmin/diosmin
16a,b Neohesperidin/ hesperidin
17 Isosakuranetin rhamnosyldiglucoside
18 Di-(3-hydroxy-3-methylglutaryl) isorhamnetin glucoside
19 Kaempferol triglucoside
21 Poncirin

Limonoids

20 Limonin

Anthocyanins

22 Cyanidin 3-*O*-glucoside
23 Petunidin rhamnosyldiglucoside
24 Cyanidin 3-(6''-malonylglucoside)
25 Delfinidin rhamnosylglucoside
26 Peonidin 3-(6''-malonylglucoside)

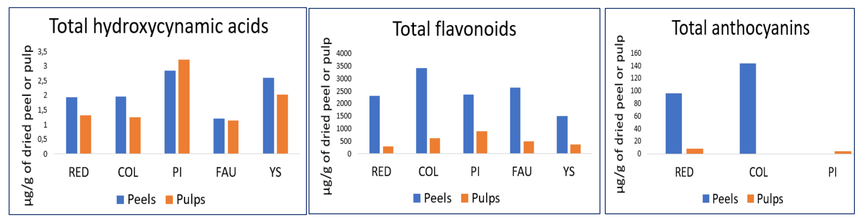


Fig.4. Metabolite amount (µg/g of dried peel/pulp).

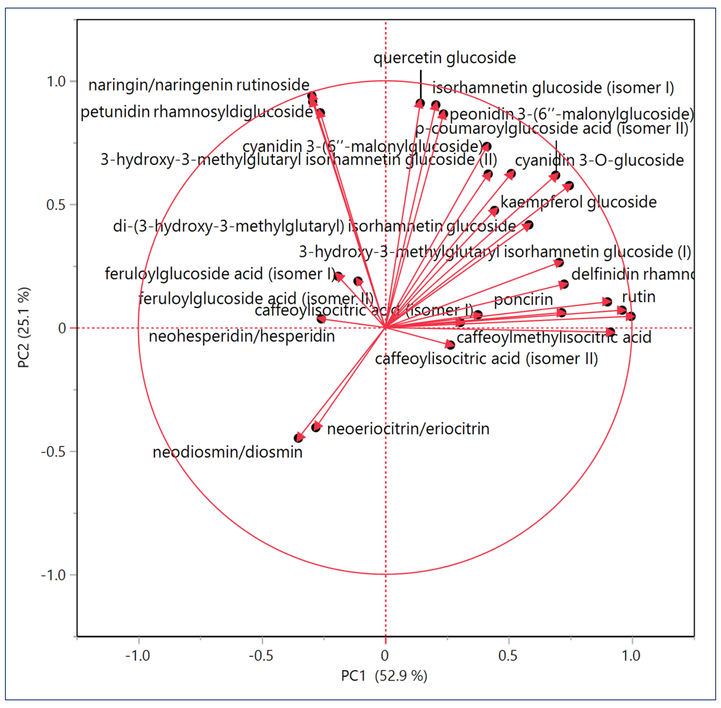


Fig.5. Loading plot of the principal component analysis (PCA).

Investigation on Thyroid Metabolism by LC-MS-MS

Moderate iodine deficiency causes gestational hypothyroxinemia in *Dehal1* KO mice

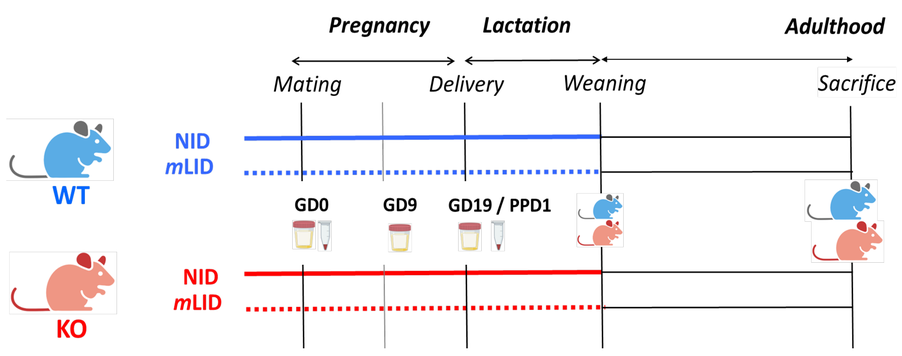
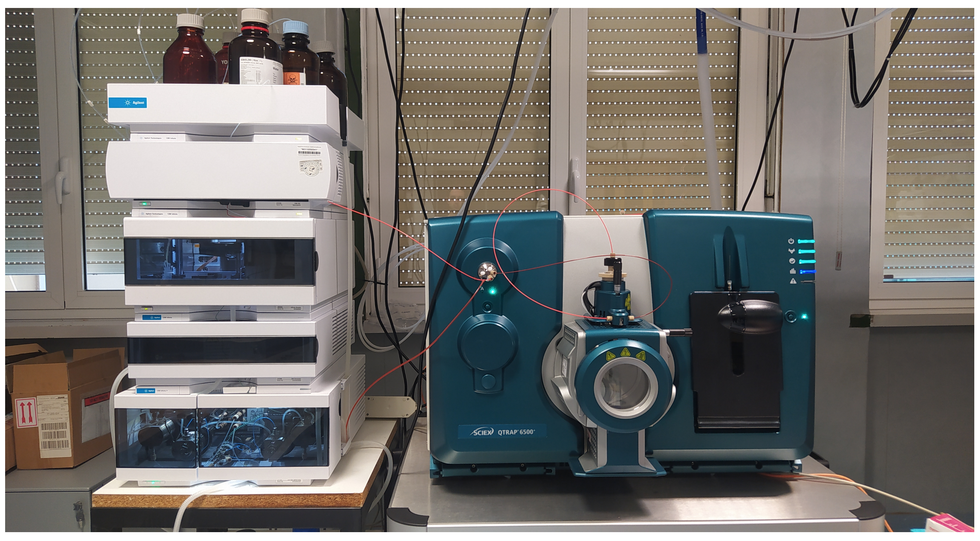


Fig.1. Experimental Setup.

Introduction: maternal hypothyroxinaemia is a pathogenic factor for alterations of fetal development, generally attributed to iodine (I^-) deficiency (ID). The enzyme DEHAL-1 is responsible for the enzymatic deiodination of TH precursors 3-iodotyrosine (MIT) and 3,5-diiodotyrosine (DIT). Its reduced activity may determine low I^- recycling and thus hypothyroidism.
Study: ID was monitored in a Dehal1-KO mice during pregnancy by a validated LC-MS/MS method, able to detect MIT, DIT, T3 and T4, in urine and plasma. WT and DEHAL-1 KO mice were fed with a normal or mild low I^- content diet (NID or mLID, 5,8 or 0.8 ug I^-/day) and monitored from mating to weaning. TH were measured in plasmas and urines collected at different timepoints.
Results: the assay showed that a mild iodine deficiency can induce a maternal hypothyroxinemia in DEHAL-1 KO mice, as mainly shown by the decrease of plasmatic T4, while T3 didn't show significant variations. Alongside with the reduction of maternal T4, plasmatic and urinary MIT and DIT decrease dramatically under iodine deficiency and remain detectable only in KO mice.
Conclusions: Dehal1 KO mice demonstrated to be a novel tool to induce maternal hypothyroxinaemia.



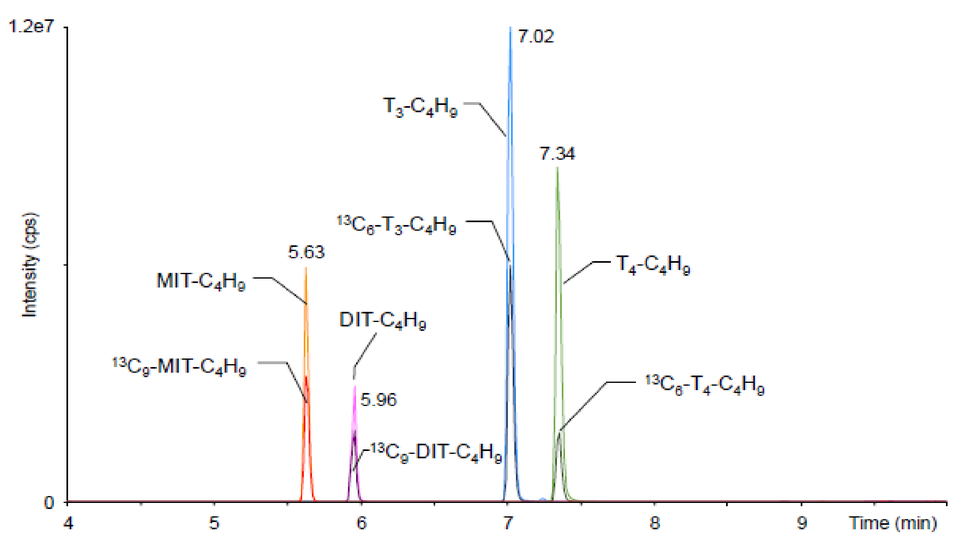


Fig.2. Instrumental layout & LC-MS/MS chromatogram of a standard solution containing butylated MIT, DIT, T3 and T4 and their relative ISs.

Sample Preparation: urine and plasma samples (100 µL) were added with appropriate amounts of isotope-labelled Internal Standards (IS), precipitated using cold acetone and dried gently under N_2. After derivatization with butanol-HCl 3.0 N to produce TH butyl-esters, samples were dried again and submitted to Solid Phase Extraction (SPE). Eluates were dried, reconstituted using an acetonitrile:HCl 0.1 M solution (50:50, v/v) and injected in the LC-MS/MS system.
Instrument layout: Agilent (Santa Clara, CA, USA) 1290 UHPLC system, including a binary pump, a column oven and a thermostated autosampler, coupled to an AB-Sciex (Concord, Ontario, Canada) QTRAP 6500+ mass spectrometer working as a triple quadrupole, equipped with an IonDrive™ Turbo V source.